The relationship between plaque accumulation and anterior crowding in an adolescent orthodontic population

Thesis submitted in accordance with the requirements of the University of Liverpool for the Degree of Doctor of Dental Science (Orthodontics)

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CONTENTS

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2. ABSTRACT

Aim: To evaluate the longitudinal relationship of anterior tooth alignment and intra-oral plaque accumulation during orthodontic fixed appliance treatment, using novel intra-oral imaging techniques.

Design: Prospective observational cohort study.

Setting: Countess of Chester Hospital

Subjects: 13 adolescent patients (9 females, 4 males) recruited prior to commencing fixed appliance therapy. The mean age of patients recruited was 13.4 years with an age range of 11.9 – 17 years.

Methods: Clinical data were collected for each participant at consecutive appointments until the anterior teeth were aligned with the minimum follow-up being 6 months. Data included an assessment of plaque accumulation using a novel hand-held device (Toothcare™) and upper and lower labial segment crowding was evaluated using sectional plaster models to determine the Little's Irregularity Index [1975].

Repeat measures were used to assess reliability of the plaque scoring method and measurement of the Little's Index via dial callipers and a reflex metrograph. The accuracy of hand scoring Little's Index was also compared to the reflex metrograph measurements. Dahlberg's method error formula was used to determine the accuracy of

Little's Index scores whilst Cohen's Kappa statistic determined the degree of agreement in repeat plaque measurements.

Uni-variate non-parametric statistics were used to assess any differences between test re-test measurements and Spearman's Correlation Coefficient was used to determine associations between plaque and crowding over time. The multi-variate Kruskal-Wallis test was used to examine the trend of plaque and crowding over time.

Results: Spearman's Correlation Coefficient showed an inverse relationship between plaque and crowding over the course of the study ($r = -0.375$) although this association was very weak. The inverse relationship was stronger for the baseline data (prior to bonding of the fixed appliances) $r = -0.602$, which was statistically significant at the five percent level.

There was no consistent trend amongst patients of plaque accumulation over time ($p=0.741$) although the degree of labial segment irregularity did consistently improve over the first three visits ($p=0.038$).

Hand scoring with dial callipers showed acceptable accuracy with a method error of 0.17mm, although the reflex metrograph did not show the same consistency (method error = 0.22mm) and tended to over score the irregularity when compared to the hand scoring method ($p=0.000$).

Reproducibility of the plaque scoring method was acceptable when using the Toothcare™ light at the chair side (un-weighted kappa statistic = 0.92) and when compared to an image from the digital camera based on the same technology, QLF-D Biluminator™ (un-weighted kappa statistic = 0.76).

Conclusions: The plaque scoring index based on a novel hand-held device (Toothcare^{TM}) shows acceptable reliability and reproducibility. Measuring Little's Irregularity Index [1975] by hand may be preferable to using a similar index on a reflex metrograph due to the increased error in the vertical dimension. Plaque accumulation does not appear to show a consistent trend over time and is very variable in nature. In this cohort there was a very weak and inverse relationship demonstrated between labial segment crowding and plaque quantity.

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4. LITERATURE REVIEW

4.1 Plaque

4.1.1 Description

Plaque is a significant aetiological factor for both dental caries and periodontal disease. It consists of a complex microbial biofilm which develops on the surface of teeth. Immediately after cleaning, tooth surfaces are coated in a ‘proteinaceous conditioning film’ known as the acquired pellicle [Dawes *et* al, 1963; Armstrong, 1966; Donlan and Costerton, 2002]. This pellicle is made up of glycoproteins, phosphoproteins, lipids, albumins and lysozyme. Following colonisation by pioneer species, a polysaccharide matrix is formed from extracellular material produced by the bacteria. The bacteria adhere to this matrix and each other to produce unique properties within the biofilm.

Existence as a biofilm can increase bacterial resistance to antimicrobial agents and can create a unique ecosystem for bacteria to thrive which would not survive in the mouth in planktonic form. [Donlan and Costerton, 2002; Thomas and Nakaishi, 2006]

Resistance to antimicrobial agents can be conferred in a variety of ways. The biofilm structure may prevent penetration and diffusion of the agent. The depth of the plaque may allow the agent to be neutralised / quenched at the surface level while the deeper microbes are still protected. Microbes within a biofilm also grow much slower and often antimicrobial agents may interfere with replication and, therefore, are more effective in fast-growing cultures. The heterogeneity of dental plaque also means that bacteria may have indirect protection via adjacent microbes and their defences with gene transfer and mutations reducing susceptibility. [Thomas and Nakaishi, 2006]

Although bacteria can adhere to most surfaces in the mouth there are certain sites where plaque build-up is more common [Marsh and Martin, 2009]:

1. The margin between the tooth and gum
2. Fissures in the occlusal surfaces
3. Approximal areas

This is because these sites provide a relatively sheltered area from the shear forces of saliva and are also areas more difficult to clean mechanically. Any surfaces which create ledges or areas of shelter (e.g. fixed appliances) are likely to increase bacterial adhesion and plaque build-up and those areas difficult to clean (e.g. in cases of tooth irregularity) will also be relatively sheltered.

The composition of plaque can vary greatly depending on the individual host and age of the plaque. Initial plaque formation involves 'pioneer' species adhering to salivary proteins and glycoproteins on the tooth surface. The main bacterial constituents of this initial plaque are streptococcal species. These initial species multiply and build up a palisade of cells which allows co-aggregation of further bacterial species. This secondary colonisation by Gram positive and negative species creates a more diverse bacterial colony until a 'biofilm' as described above is formed. The exact composition of the plaque varies according to environmental factors such as availability of fermentable carbohydrates, pH and site. [Marsh and Martin, 2009]

4.1.2 The role of plaque in oral disease

There have been various theories in the past as to how microbes within plaque are implicated in disease. According to the German Physician Robert Koch, for a specific pathogen to be implicated as part of a disease process it should fulfil his four criteria: [Koch, 1890]

1. The microbe should be found in all cases of the disease with a distribution corresponding to the observed lesions.
2. The microbe should be grown on laboratory media for several subcultures.
3. A pure subculture should produce the disease in a susceptible animal.
4. A high antibody titre to the microbe should be detected during infection; this may provide protection on subsequent reinfection.

After much research it has not been possible to identify a single microorganism which completely fulfils Koch's postulates in relation to plaque-mediated diseases. The complication comes from the fact that while key organisms seem to be implicated in causing disease, they have also been found at healthy sites. [Takahashi and Nyvad, 2008]

The recent concept of the role of plaque microbes in relation to disease is an amalgamation of the specific and non-specific plaque hypotheses which suggests that there are specific organisms associated with disease, but that they have to be present in sufficient numbers within a favourable environment to initiate disease. [Thomas and Nakaishi, 2006] This environment depends on various factors such as age, oral hygiene, diet, smoking and genetic variations in inflammatory response of the host. This is known as the ‘ecological plaque hypothesis’. [Marsh and Martin, 2009]

4.1.3 Role of plaque in enamel demineralisation

The role of bacteria in dental caries was initially proven through experimentation with germ-free rodents. Even when fed a cariogenic diet these rodents did not develop caries unless they were infected with streptococci bacteria. These experiments also highlighted the importance of diet in caries aetiology as another pre-requisite for the development of a lesion was the presence of sucrose. [Marsh and Martin, 2009]

The accepted explanation for the aetiology of caries is based on W.D. Miller’s ‘chemico-parasitic’ or ‘acidogenic’ theory which he published in 1890. This theory postulates that it is acid produced by plaque bacteria fermenting dietary carbohydrates that leads to decalcification of teeth and this can subsequently lead to degeneration of the organic matrix by proteolytic bacterial action.

Epidemiological studies have helped to show a strong association between the presence of *mutans streptococci* and the initiation of smooth surface and fissure caries, whilst *lactobacilli* may contribute but not induce carious lesions [Tanzer *et al*, 2001]. Within orthodontics the main area of interest is enamel demineralisation or ‘white spots’ which are characteristic of the initial stage of caries prior to cavitation. Gorelick and co-workers [1982] suggested that up to 50% of patients undergoing fixed appliance therapy developed white spot lesions during treatment. Although Mizrahi’s [1982] similar cross-sectional survey suggested an incidence for new white spot lesions of only 12% following orthodontic fixed appliance treatment.

Acidogenic plaque bacteria (in particular the *mutans streptococcal* strains) generate acid when metabolising sugars. This acid can then dissolve the mineral phase of enamel only if the pH falls below a critical level of pH5.5 [Marsh and Martin, 2009]. These bacteria are also often aciduric and a cycle of increasing colonisation by acidogenic bacteria occurs the more frequent the intake of fermentable carbohydrates and the lower the pH [Takahashi and Nyvad, 2008].

Demineralisation and remineralisation are dynamic processes and dependent on the gradient of ion saturation. Whilst demineralisation dominates when the pH falls below a critical level it is possible to remineralise enamel once the pH rises again. The process of remineralisation is further aided by the use of fluoride ions which can substitute for hydroxyl ions to create fluorapatite which is more resistant to dissolution and can create a more stable crystal lattice. The presence of aciduric bacteria such as mutans streptococci creates an aciduric environment which increases the number of acid producing bacteria and causes enamel lesions to develop as the environment is shifted to 'mineral loss'.

As well as the presence of plaque bacteria there are other factors which influence the risk of developing a demineralised lesion; such as frequency of carbohydrate intake which will increase the period of time the pH falls below the critical level and, therefore, favour demineralisation; and the type of carbohydrate, with monosaccharides being much quicker to metabolise than more complex carbohydrates [Banerjee and Watson, 2011]. It is the creation of 'pores' in the enamel as a result of demineralisation which gives the characteristic white appearance of early enamel caries which is the type most often associated with orthodontics.

4.1.4 Role of plaque in periodontal diseases

Epidemiological and experimental studies have been able to show a strong association between the presence of dental plaque and periodontal diseases [Loe *et al*, 1964]. There are two main types of periodontal disease: gingivitis which is not associated with irreversible destruction of the periodontal tissues and periodontitis which is associated with periodontal attachment loss [Armitage, 1999]. Gingivitis can further by divided into plaque-induced and non-plaque-induced conditions. Work by Socransky and colleagues [1998] identified five 'complexes' of plaque bacteria and the more virulent organisms, closely associated with clinical signs of periodontal diseases including bleeding on probing and pocket depth, tended to be anaerobic bacteria [Socranksy *et al*, 1998], particularly those identified as part of the 'red complex': *Porphyramonas gingivalis, Bacteroides forsythus and Treponema denticola*. This suggests that periodontal diseases are polymicrobial infections.

Whilst the presence of these specific plaque bacteria plays a major aetiological role in periodontal disease, the severity of infection can vary considerably according to host factors. Periodontal disease is the inflammatory reaction to infection and the degree of damage (periodontal and bone) is modulated by the degree of host response and ability to control the infection. Similarly to caries pathogenesis the process of the disease tends to create an environment which favours the pathogenic bacteria (acidogenic in caries and anaerobic in periodontal disease). The hypothesis is that inflammation results in an increased flow of gingival crevicular fluid with an increase in pH and temperature which favours the growth of proteolytic obligate anaerobic species associated with periodontal diseases [Marsh and Martin, 2009]

4.1.5 Methods of detecting and measuring plaque

Visual Detection

Early plaque forms a very thin layer and is invisible to the naked eye. Even as layers of plaque build it can be very difficult to detect with the naked eye alone and this may explain the reason behind many of the traditional plaque indices whereby probes may be used to detect the presence of plaque if not obvious by sight alone. In an effort to improve detection and quantification of plaque, methods of enhancing visualisation of the plaque biofilm have been developed.

Disclosing Tablets

In order to make plaque deposits more visible to the naked eye a group of dyes have been developed which preferentially stain plaque. The most common dye used is erythrosine which stains plaque material together with the acquired pellicle red. There exists a variety of other disclosing agents which also increase the visibility of the plaque deposits. One method uses a product containing fluorescein (Plak-lite™) which is colourless when applied but fluoresces under filtered white light [Boyd, 1983]. Whilst this study found disclosing agents to be advantageous in aiding oral hygiene in patients other studies stress the importance of their use in conjunction with adequate verbal instruction in the clinic [Tan and Wade, 1980]. As well as increasing the visibility of plaque, there are agents available which are able to differentiate mature and recent deposits of plaque according to the dye uptake, which is important as mature plaque may be more aciduric [Pretty *et al*, 2005].

Whilst methods of disclosing plaque help to visualise the distribution of plaque, it is often important in research to quantify the amount of plaque present, usually as an expression of the area of tooth surface coverage. This may be done in conjunction with disclosing if needed. The following section will discuss the various methods of quantification of plaque deposition.

Plaque Indices

An index uses numerical values to describe the relative status of a feature on a scale with a defined upper and lower limit [Russell, 1967]. Using carefully defined indices can allow comparison of the severity of a disease or attribute between different population groups. A good index should be both reliable and valid, however, many indices are subject to variations in examiner subjectivity. Reliability is the consistency of the measurement on multiple occasions and validity is the capacity of a test, instrument or question to give a true result [Bruce *et* al, 2008]. Analysis of data can also be complicated by the fact that most indices use an ordinal scale and require non-parametric analysis [Lang *et al*, 1998].

There are many different indices available and the choice of which one to use depends on the objectives of the trial, the size of population studied, the length of the trial and the magnitude and type of change expected [Fischman SL, 1986]. Most plaque indices measure the amount of tooth surface covered, although some assess the thickness of plaque present and are non-linear indices and should be analysed accordingly.

One of the initial plaque indices developed as part of the periodontal disease index by Ramfjord in 1956 focuses specifically on the gingival half of the interproximal tooth surfaces. This is because plaque here is more relevant to the development of periodontal diseases than coronal plaque [Ramfjord, 1959]:

0 = Absence of dental plaque

1 = Plaque present on some, but not all, of the interproximal and gingival surfaces of the tooth.

2 = Plaque present on all interproximal and gingival surfaces, but covering less than one-half of the entire clinical crown.

3 = Plaque extending over all interproximal and gingival surfaces, covering more than one-half of the entire clinical crown.

The total score is divided by the number of teeth examined to determine a mean score per tooth.

Another index developed at the same time was the Oral Hygiene Index [Greene and Vermillion, 1960]. This incorporated an index for calculus deposits as well as soft plaque deposits. The plaque or 'debris index' is described below:

0 = No debris present.

1 = Soft plaque debris covering not more than one-third of the tooth surfaces being examined.

2 = Soft plaque debris covering more than one-third but less than two-thirds of the exposed tooth surfaces.

3 = Soft plaque debris covering more than two-thirds of the exposed tooth surfaces.

The total debris score for all teeth is divided by the number of surfaces scored to give an oral cleanliness score. The oral hygiene is termed 'good' if the score is 0.3-0.6, 'fair' when 0.7-1.8 and 'poor' if 1.9-3.

Another reliable index used for estimating the area of tooth covered by plaque is the Quigley and Hein Index [1962] which was modified by Turesky and colleagues [1970]. This technique is often used when evaluating the effectiveness of anti-plaque procedures and a diagrammatic representation is shown below [Figure 4.1]:

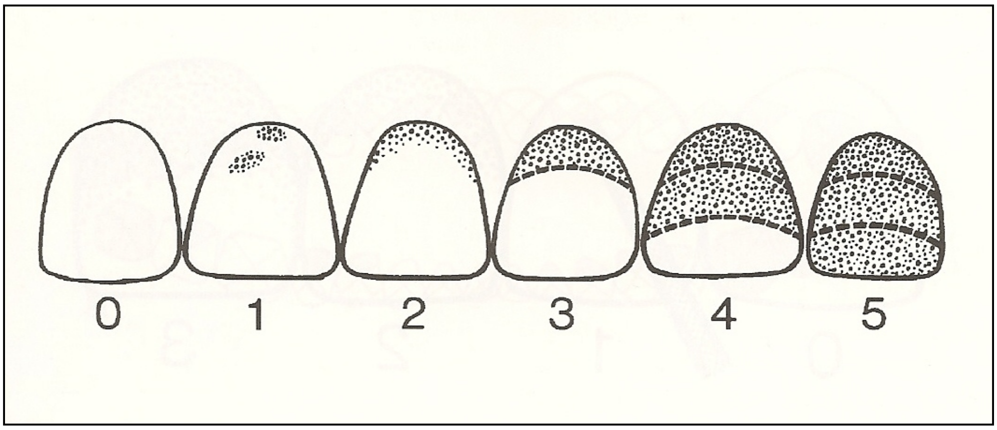

Figure 4.1: Illustration of Quigley Hein Index [1962] reproduced from Lang *et al*, 1998. Shaded areas represent degree of plaque coverage for each score on the index.

0 = No plaque

1 = Separate flecks of plaque at the cervical margin of the tooth.

2 = A thin continuous band of plaque (<1mm) at the cervical margin.

3 = A band of plaque wider than 1mm but covering less than 1/3 of the crown.

4 = Plaque covering 1/3 – 2/3 of the crown.

5 = Plaque covering 2/3 or more of the crown.

Again an average score per tooth is recorded. The Turesky modification [1970] includes assessment of the lingual as well as buccal surfaces of all teeth.

In 1967 O'Leary developed a score which evaluated buccal and lingual surfaces of all teeth present and involved adding the highest scores in each segment and dividing by the number of dentate segments:

0 = No plaque on any tooth in the segment.

1 = A slight amount of plaque not extending 2mm from the gingival margin on any tooth in the segment.

2 = Plaque covers up to 50% of the exposed clinical crown on any tooth in the segment.

3 = Plaque covers more than 1/3 of the clinical crown.

Disadvantages of this index are the tendency to over score the plaque at the incisal half of the crown as opposed to that at the gingival margin.

Following on from this O'Leary and colleagues [1972] developed a further index. This was a simplified method with a dichotomous scoring system for each tooth surface (simply marked for presence of absence of plaque). Each tooth is divided into 4 sections: buccal, lingual, mesial and distal. After the teeth have been disclosed, the number of surfaces with plaque present is marked on a full mouth charting. The total sum of surfaces positive for plaque presence is calculated and divided by the total number of surfaces present and multiplied by 100 to provide a percentage plaque score for the full mouth. An example of a chart used is shown in Figure 4.2 below:

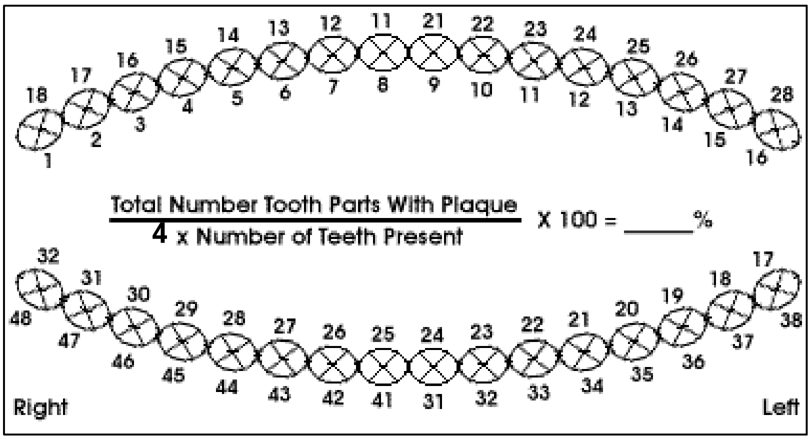

Figure 4.2: O'Leary Plaque chart reproduced from Lang *et al*, 1998. The division of each tooth surface and how a full mouth percentage plaque score is calculated is illustrated.

Another common index is one developed by Silness and Löe in 1964 which is generally used without needing to disclose plaque present:

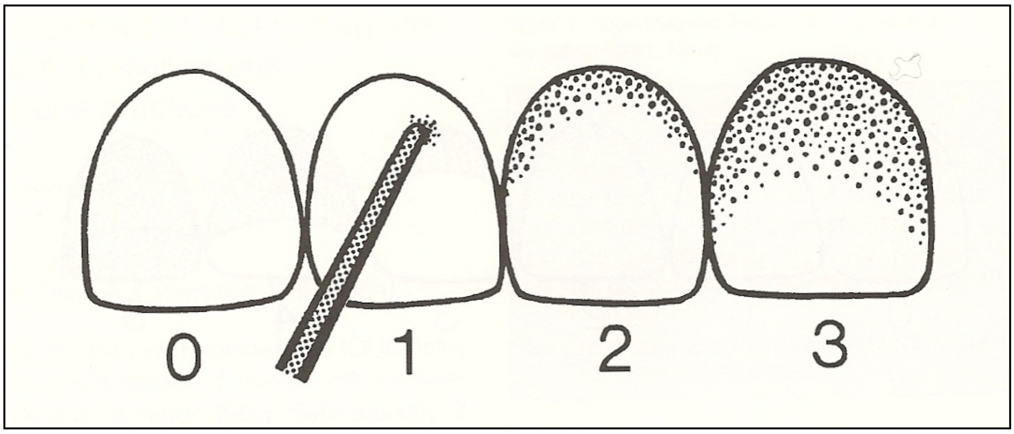

Figure 4.3: Illustration of Sillness and Löe Index reproduced from Lang *et al*, 1998. Shaded areas represent plaque deposits.

0 = Gingival area of tooth surface free of plaque tested by running probe across tooth.

1 = No plaque initially observed and only visualised after probe run across tooth.

2 = Gingival area is covered with a thin to moderately thick layer of plaque visible to the naked eye.

3 = Heavy accumulation of soft matter, the thickness of which fills out the niche produced by the gingival margin and the tooth surface.

This index only considers differences in thickness but not area of tooth surface covered by plaque and focuses on plaque accumulation at the gingival margin. Whilst this may be relevant for periodontal disease, it underestimates the coronal extension of plaque which may be implicated in demineralisation. A disadvantage of this method is its subjectivity and that it can only be carried out once as plaque removed, therefore, it is not possible to use multiple examiners.

Another dichotomous scoring method is the patient hygiene performance index [Podshadley and Hayley, 1968] which was suggested for use to evaluate a patient's ability to follow toothbrush instructions. It is carried out after the use of a disclosing solution and the buccal surface of each tooth is divided into 5 sections. The presence of any plaque in a given section is scored 1 (absence of plaque scores 0) and the total is summed and divided by the number of surfaces examined to give a PHP score.

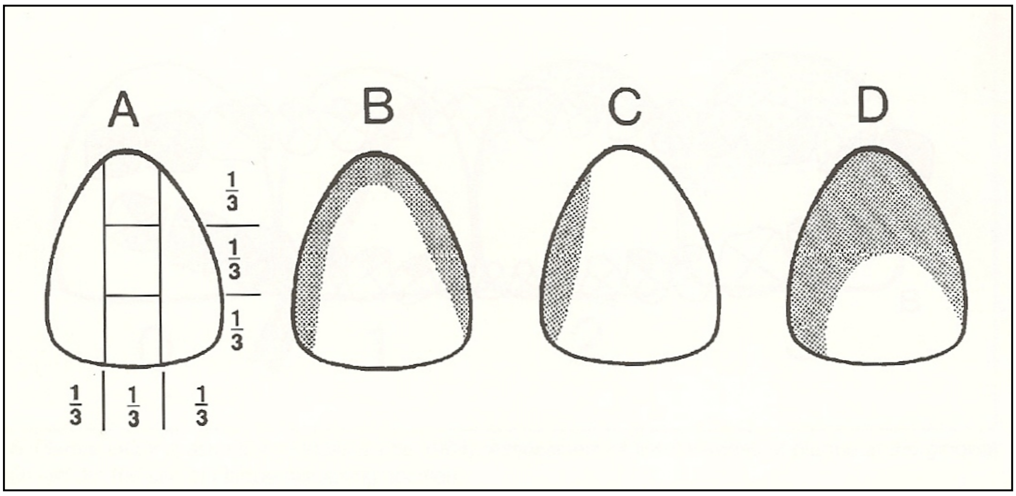

Figure 4.4: Patient Hygiene Performance Index [Podshadley and Hayley, 1968] A:5 subdivisions B: Plaque score of 3 C: Plaque score of 1 D: Plaque score of 4

As mentioned previously, plaque accumulated at gingival margins has more importance in periodontal disease and as a result the Navy system [Eliot *et al*, 1972] gives greater weighting to gingival plaque whilst still assessing the full tooth surface. Each surface is divided into sections as shown below there are three sections in the gingival area, 2 in the middle and one occlusal section. If plaque is present a score of 1 is awarded, absence is not scored (similar to the previous index):

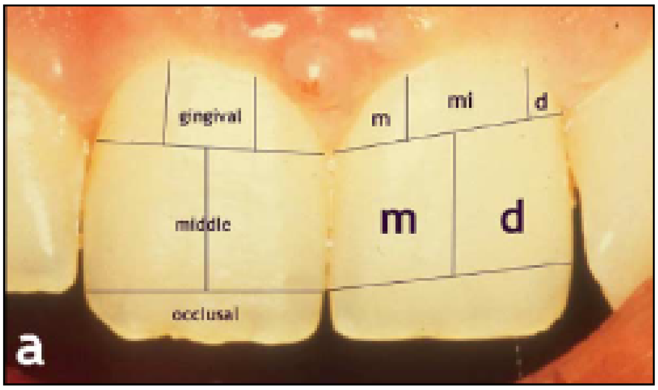

Figure 4.5 Diagram of 'Navy' scoring system [Pretty *et al,* 2005] illustrating sections of each tooth to be examined with a dichotomous score of 1 if plaque is present or 0 if there is none.

As already mentioned all of these indices are based on integer scales rather than a direct quantification of the amount of debris present. Also in many indices, there is a greater emphasis on plaque deposits at the gingival margin - this may be useful for periodontal screening but may not be helpful when assessing an orthodontic population as the whole tooth surface must be assessed. As can be seen there are many plaque indices and each may be suitable for different situations, however, the two indices recommended for common use in clinical oral hygiene trials [Council on Dental Therapeutics, 1985] are the Silness and Löe Index [1964] and the Turesky modification of the Quigley-Hein Index [1970]

Planimetric measurements

This method involves the use of photographic images to determine the Plaque Percentage Index (PPI) which relates to the percentage area of the tooth covered by plaque. This requires disclosing teeth and then recording photographic images which can by scored either by hand tracing or by computer analysis using a count of pixels [Pretty *et al*, 2005]. This allows the quantification of plaque on an interval scale as opposed to the discrete integer scale of traditional indices. In this way, it may make it more sensitive for measuring small changes in plaque levels.

The Stain Index described by Shaw and Murray [1977] describes a process of tracing the plaque distribution on a scaled up outline of a tooth surface (4 times magnification) and counting the number of 4mm squares shaded in to determine the surface area of plaque coverage. This is more subjective than computer analysis as there is potential for error in transferring the distribution of plaque accumulation to the map of the tooth. The Stain index has been used in the cross-sectional study by Griffiths and Addy [1981] which was looking at the effects of the malalignment of teeth in anterior segments on plaque accumulation.

Whilst studies have found planimetric methods to deliver excellent intra-examiner and inter-examiner reliability and the computer based methods particularly prove to be more precise, objective and sensitive than traditional indices, it is time consuming and can be expensive [Söder *et al*, 1993]. In addition, as it is a 2-dimensional representation it can only be used to determine plaque area and not depth. It is also important to produce reproducible photography which may require the use of positioning jigs and may tend to restrict analysis to buccal surfaces of the teeth. An advantage of this technique, however, is that the images can be stored and used again for multiple trials.

A modification of this technique is to use fluorescein dye (as mentioned previously) instead of the traditional erythrosine disclosing solution. This does provide a more sensitive measure of the area of plaque present as fluorescein disclosed plaque is a significantly different colour from the surrounding tissues, which can be a problem with erythrosine disclosing. A subjective assessment has to be made of which area is plaque and which is disclosed soft tissue. A disadvantage of the method is that fluorescein works best at a low pH which poses a demineralisation risk to teeth, therefore, the use of a phosphate buffer following rinsing with the solution is recommended [Pretty *et al*, 2005]. An example of the images obtained is show in Figure 4.6 below.

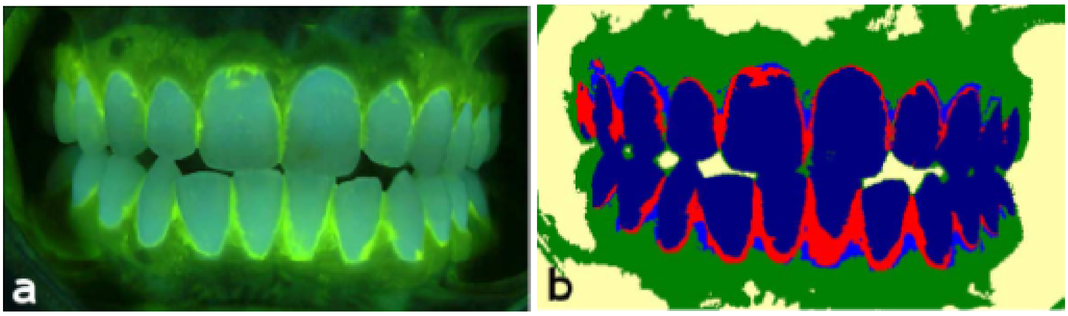

Figure 4.6: a: Digital photograph with fluorescein stain b: Image analysis displaying clear demarcation of plaque and tooth surfaces.

Quantitative Light-induced Fluorescence (QLF)

This was developed as a method of diagnosing carious lesions as it is a sensitive measure of the degree of mineralisation of dental enamel. In this way QLF is a very useful tool for longitudinal monitoring of early enamel caries and its progress or regression. [van der Veen *et al*, 2006] It is possible to use this tool in both the clinical and lab setting and it has been developed for research and clinical application.

QLF is based on the natural fluorescence of teeth with carious lesions showing less fluorescence than sound enamel [Angmar-Mänsson and ten Bosch, 2001]. It is based on an arc lamp which passes through a blue filter to produce a peak intensity of light of 370nm. Any reflected fluorescence is then passed back through a yellow high pass filter of 520nm and this combination of blue and yellow light helps to minimise reflections (a common problem with white light images of demineralisation). This then provides quantitative data for the area of demineralisation and the mean loss of fluorescence [Benson P *et al*, 2003].

Investigations to determine the optimal conditions for using the QLF system noted that heavy deposits of plaque would fluoresce either deep red, bright orange or green in colour [Amaechi and Higham, 2002]. This led to the recommendation of prophylactic cleaning of teeth when utilising QLF to monitor enamel demineralisation. It is thought that the fluorescence is caused by bacterial products which are chromophores of porphyrins [Konig et al 1993].

The colour of autofluorescence of the plaque sampled may further indicate the age of the deposits. In a study involving denture plaque by Coulthwaite and colleagues in 2005, they determined that red and orange isolated plaque deposits were particularly associated with more mature plaque and examples of species isolated were *F. Nucleatum* and *Prevotella species* which are commonly implicated in periodontal disease [Coulthwaite *et al*, 2005]. Green fluorescence, however, tended to indicate a ‘younger’ plaque colony and was related to pioneer species such as streptococcal bacteria.

Another study carried out in Amsterdam suggested that red fluorescing plaque comprised only about 62% of the total plaque which was disclosed and that red-fluorescing plaque tended to accumulate at the gingival margins (or plaque stagnation sites). This study suggested that only anaerobic bacteria display auto-fluorescence and examples were *A. odontolyticus* and *Prevotella intermedia*. In addition even greater red-fluorescence was detected when *P. gingivalis* and *P. micros* were grown in close proximity. [van der Veen et al, 2006]. This led the authors to conclude that bacteria existing in a biofilm may fluoresce differently than in isolates and that it is the synergistic activity of neighbouring bacteria which contributes to strong red auto-fluorescence. They also extrapolated their findings to suggest that red fluorescence is more likely to be seen with a mature biofilm.

The advantage of QLF over previously mentioned methods of plaque detection is that the small camera can provide reproducible images which are free from distortion and reflection which can be a problem associated with planimetric diagnosis using white light photographs. It is also proposed that QLF is more sensitive than disclosing plaque and, therefore, small changes in plaque volume should be detected more easily. [Pretty *et al*, 2005]

Toothcare™ - Developed by Inspektor Research Systems, Amsterdam

This is a novel hand-held device based on the same principles as QLF but without the ability to capture images. It also emits a blue light but in order to make the device more portable it is not connected to a control unit and monitor. Light is emitted from a 405nm blue LED and when used in conjunction with goggles which filter the yellow and red light reflected (with transmission peaks around 500nm and 630nm) it allows the operator to visualise areas of fluorescent plaque and demineralisation at the chair side [van Daelen *et al*, 2008]. The results of an MDentSci study into the validity of this method show it is comparable to traditional QLF methods for plaque detection. [Thomas, 2010] Below is a picture of the device:

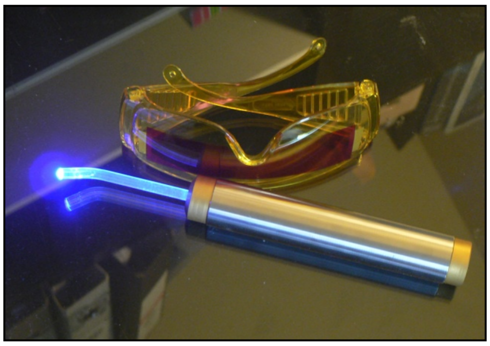

Figure 4.7: Toothcare™ device – Inspektor Research Systems, Amsterdam. Hand held device which emits blue light, the operator wears the safety goggles illustrated with a red filter to identify areas of fluorescent plaque accumulation.

<u>QLF- D Biluminator™</u> – Developed by Inspektor Research Systems, Amsterdam

Again, based on the technology behind QLF and Toothcare™, a digital camera has been developed which records an image similar to that seen with the hand held Toothcare™ light. The device combines an SLR camera with filters and light sources to create a set of pictures in the clinical environment. Each record produces a traditional white light image and a QLF type image which illustrates bacterial porphyrins and enamel demineralisation.

Fluorescent plaque or calculus shows up bright red in the QLF image, although as discussed previously only plaque that has been present for some time (longer than 1 day) will fluoresce red. Enamel demineralisation appears as a shadow in the image due to the reduced light reflection caused by porosities in the enamel. Below is an example of the images obtained from the QLF-D Biluminator™:

Figure 4.8: Example of QLF (left) and white light (right) images from QLF-D Billuminator with indicating areas of plaque deposition and relative differences in detecting plaque between the two images.

4.2 Crowding

4.2.1 Description

Dental crowding is the most common cause of malocclusion and can be associated with any of the underlying skeletal jaw relationships. It is thought to have an incidence of 60% amongst Caucasians [Todd and Dodd, 1985]. There may be many reasons why crowding arises such as a tooth size / jaw size discrepancy, mesial movement of posterior teeth resulting in a shorter arch length and retroclination of the lower incisors which also shortens the arch length.

It is, therefore, essential to have methods of measuring the degree of crowding to determine greatest need for treatment. There are various methods that have arisen; the simplest is to measure the discrepancy between space available within the arch and the space required for the teeth present. Whilst this provides us with an accurate quantification of the amount of crowding present, it is not descriptive regarding the degree of irregularity amongst the teeth. For example 3mm of crowding localised to one tooth will display a large degree of irregularity / discrepancy whereas if this is spread between the 6 anterior teeth the irregularity is greatly reduced.

4.2.2 Measurement methods

<u>*Malalignment Index*</u>

This index has been used in previous studies regarding the relationship between dental irregularity and periodontal disease [Ainamo J, 1972; Buckley, 1981]. It was developed by van Kirk and Pennell as a method of measuring individual tooth irregularity [1959]. This is an ordinal index rather than a quantitative measure of irregularity:

0 = Ideal alignment of incisors with the tooth showing no apparent deviation from an ideal line projected through the contact points.

1 = Minor mal-alignment of rotation or displacement:

Line projected through the contact points is less than 45^{o} to the ideal arch.

Both contact points displaced from the ideal arch but by less than 1.5 mm.

2 = Major mal-alignments with rotations greater than 45^{0} or more than 1.5 mm of incisor displacement from arch

This method, therefore, does not give a continuous quantification of tooth irregularity and would be unable to differentiate between moderate and severe cases of displacement.

In Buckley's cross-sectional study [1981], he also used a second measurement of lower anterior arch crowding which forms part of the Occlusion Feature Index (OFI) of Poulton and Aaronson [1961]. This Index also involves measurements of cuspal interdigitation, vertical overbite and horizontal overjet to give an overall score for the malocclusion which can range from 0-9. The categories for lower incisor crowding are listed below:

0 = No crowding

1 = Crowding of the lower anterior teeth equivalent to one half the width of a central incisor

2 = Crowding between one half to a full width of a lower central incisor

3 = Crowding exceeding the width of one central incisor

There are many other methods which provide a subjective assessment of incisor crowding as merely present or absent (with any incisor displacement greater than 1mm) whilst others may subjectively rank severity on a scale from 0-5 such as the Proffit-Ackerman rating scale [1973]. The different grades are not based on a quantitative measurement of the crowding but related to the clinical implications relating to orthodontic treatment as this scale was intended for use in aiding treatment planning as opposed to use in epidemiological studies:

0 = Ideal, no deviation

1 = Slight – deviation from ideal but patient would not require treatment based on this characteristic alone.

2 = Slight – moderate

3 = Moderate – this deviation alone would justify treatment

4 = Moderate – severe

5 = Severe – the patient is handicapped because of this deviation

Little's Irregularity Index

This was developed by Robert Little in 1975 in order to provide a quantitative, objective measure of anterior incisor irregularity. The method involves 'measuring the linear displacement of the *anatomic* contact points' [Little RM, 1975]. This is measured for each of the incisor contacts resulting in 5 measurements (from mesial point of canine to canine) which when combined give a score representing the degree of anterior dental irregularity.

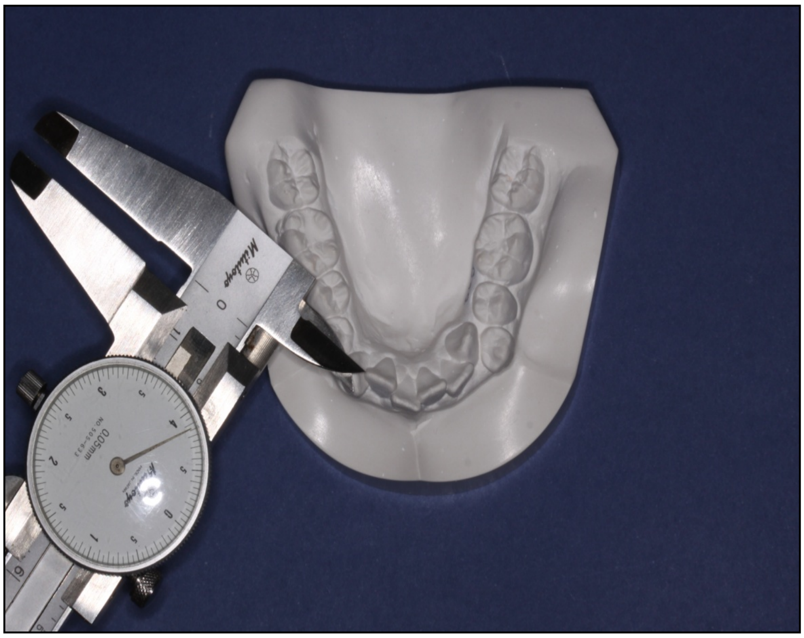

Figure 4.9: Illustration of dial callipers being used to measure the horizontal contact point displacement between lower incisors as part of Little's Irregularity Index

Little advised using a dial calliper for accuracy to 0.1mm and that the measurements must be taken from study models as it is important that the calliper is parallel to the occlusal plane when taking measurements. The method is purely a measurement of horizontal discrepancy, not vertical displacements, however, Little explains this by suggesting that vertical discrepancies rarely have an appreciable effect on anterior arch length.

Little's own study in 1975 has proven this method to have good reproducibility and consistency within examiners as well as a greater accuracy and validity than more subjective measures of irregularity. He does also acknowledge the tendency of the index to exaggerate cases with considerable irregularity but little arch length discrepancy (the higher score appearing to suggest the cases are harder to treat than they are in reality). For the purpose of this study, however, the degree of irregularity is paramount and the index is not being used to determine treatment complexity.

Reflex Metrograph – Developed by Ross Instruments Ltd, UK

This is not a method of measurement, rather a tool to enable more accurate quantification of the irregularity index. The instrument consists of a corrected semi-reflecting mirror and a movable mark with a 0.3mm diameter pin-point light spot. It is possible to move the light spot within 3 planes of space. The observer views the object's reflection in the mirror and the light spot can be moved to lie over the point of interest. When the light spot is visually co-incident with the point on the object it is exactly the same distance behind the mirror as the object is in front. The rack carrying the light marker provides co-ordinate data in 3-dimensions via rotary encoders mounted on the framework. These co-ordinates can then be used to measure linear, angular and volumetric dimensions of the object being measured.

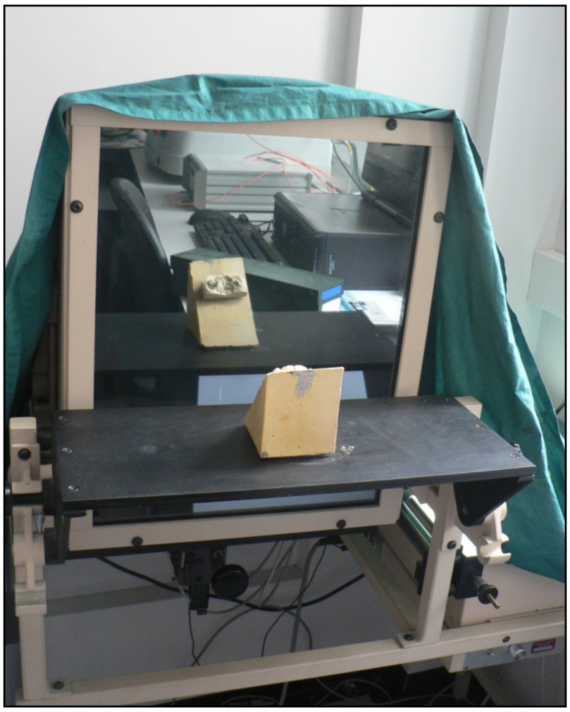

Figure 4.10: Reflex Metrograph with sectional dental cast mounted in front of the silvered mirror on an angled block to allow measurement of contact point displacements.

It has been shown that a reflex metrograph can produce precise and reproducible measurements within an accuracy of 0.2mm for linear distances [Speculand *et al*, 1988]. A certain amount of calibration has to be carried out, however, for the operator to produce such reproducible results. Within the context of this study the Reflex Metrograph was used as a secondary measurement of Little's Index in order to determine whether there is any systematic error in the measurement of the study models. If there is no systematic difference between hand and instrument measurement the secondary measures could also help to quantify random error of measurement.

4.3 The relationship between crowding and plaque

It would seem reasonable to suggest that crowded teeth would impede oral hygiene and therefore predispose to periodontal / demineralisation problems and that treatment with orthodontic appliances should help reduce this susceptibility. Evidence so far, however, has proven equivocal with only minor evidence to support the hypothesis that improved dental alignment reduces plaque accumulation [Van Gastel *et al*, 2007]. The great variation in results is possibly due to various methodological differences, often in the instruments used to measure irregularity or plaque accumulation. The majority of studies have also concentrated on the role of plaque deposits in periodontal disease rather than as an aetiological factor in enamel demineralisation.

One of the earliest studies was carried out by Ainamo in 1972 on a group of 152 army recruits aged 19-22 years. This was a cross-sectional study which used the Van Kirk and Pennel index [1959] for tooth irregularity and the Silness and Loe Plaque Index [1964]. Both of these are ordinal indices open to a large amount of subjectivity in measurement. The results of this study suggested that mal-aligned anterior teeth may be an aggravating factor for plaque accumulation as significant differences in plaque accumulation were seen as the degree of incisor irregularity increased. This was not the case for posterior molars and premolars. The conclusion from this study was that at the extremes of oral hygiene competency (i.e. excellent or very poor) the effect of crowding was masked. Ainamo [1972] suggests that it is still possible for a motivated patient to achieve excellent oral hygiene of mal-aligned teeth and that if oral hygiene is poor this will overpower the effect of crowding. An advantage of this study is that the author subdivided the results to different regions of the mouth avoiding the effect of ‘whole mouth’ means which would mask effects of irregularity which is mostly seen anteriorly. The results of this, however, would have been affected by the ordinal scale used to assess plaque accumulation which does not allow detection of small changes in plaque and the cross-sectional design only allows inferences about associations to be made but does not allow determination of a concrete relationship.

Ingervall in 1977 carried out a prospective cohort study of 50 dental students displaying varying degrees of crowding. Crowding was determined from dental casts where teeth were displaced 2mm from the arch or there was at least 15^0 of rotation. Again, the Silness and Loe Plaque Index [1964] was used and each participant had plaque index, gingival index, pocket depth and alveolar bone level recorded for a ‘crowded’ site and a contra-lateral control site (showing no crowding). The results of this study suggested that there was an equal increase in plaque levels in both groups and that the presence of crowding did not enhance plaque accumulation. Again, however, the measure of irregularity was not very sensitive and did not differentiate between variations in amount of irregularity.

The majority of studies, even those showing a small correlation of plaque to crowding, use the Silness and Loe [1964] ordinal scale for measuring plaque. While this scale allows determining the presence or absence of plaque, it does not give a sensitive, quantitative measure of the plaque present [Ngom *et al*, 2006]. The majority of previous studies also fail to describe any method of enhancing visualising the plaque present and this could lead to underestimating levels. This is highlighted in the 1998 Ashley study which showed an association between tooth irregularity and gingivitis but no significant correlation between irregularity and plaque accumulation. This was a cross-sectional study involving 201 schoolchildren examined by two researchers for evidence of gingivitis, irregularity and plaque accumulation of their 8 incisors. Irregularity was measured in mm and specified if there was overlap or spacing of the teeth. Three plaque scores were measured: the Silness and Loe Index [1964], a dichotomous measure of visible plaque and estimated dry weight of the plaque [Ashley *et al*, 1998].

Despite finding a correlation between irregularity and gingivitis scores, there was no clinically significant relationship found between the amount of plaque accumulation and the degree of tooth irregularity. The authors suggested that where there is crowding, the overlap would make it difficult to accurately assess the amount of plaque present thereby underestimating the levels of plaque and possibly diluting further any differences present. They cite this as a major reason why their study failed to find an association between crowded teeth and plaque levels. Other possible explanations put forward for the results included the mal-alignment altering the gingival contour and predisposing the subject to gingivitis or that gingivitis is not related to the amount of plaque present but the type of plaque (i.e. age of the plaque biofilm).

Whilst the previous studies have not elicited a relationship between plaque levels and tooth irregularity, there are some which have found weak to strong correlations and some of these will be discussed here.

Much of the work cited was carried out in the 1980s and again, like those previously mentioned, varied methodologically producing inconsistent results. The majority are cross-sectional studies again only providing a snapshot view which does not necessarily allow strong correlations to be determined. The first by Buckley [1981] was carried out on a group of 300 subjects and used the Van Kirk and Pennell Index [1959] as well as a summary index of degree of malocclusion [Poulton and Aaronson, 1961]. Buckley concluded that there is a positive correlation between irregular teeth and an increase in plaque accumulation, however, he was also careful to caution that this may be a more important finding in patients with poor oral hygiene.

Another cross-sectional study this time by Behlfelt and colleagues [Behlfelt *et al*, 1981] was based on a much smaller sample of 30 patients. This study, however, used each participant as their own control, similar to the Ingervall [1977] study, in an attempt to control for confounding factors such as socio-economic status, frequency of tooth brushing, time of day etc. The sample only looked at either mal-aligned lateral incisors or second premolars. They developed their own 'Malposition Index' to describe the displacement of teeth in four aspects: horizontal, vertical, crowding and rotation or tipping and the final score was classed as no mal-alignment, mild, moderate or severe. They did find a statistically significant relationship between irregularity, plaque and gingivitis. There are certain aspects, however, which may have weighted the study in this direction. They only looked at a very restricted number of teeth which may be more liable to different amounts of plaque accumulation (not just relating to mal-alignment). They also only analysed data where there was evidence of visible plaque which again would skew the results to finding a relationship as those sites with mal-alignment and no visible plaque were excluded. The very extensive mal-alignment index may have tended to overestimate the degree of irregularity present due to double scoring where certain parameters overlap, for example the horizontal discrepancy and degree of local crowding.

Another study supporting a relationship between plaque and irregularity is that by Griffiths and Addy which was another cross-sectional study measuring mal-aligned and control teeth within each individual participant [Griffiths and Addy, 1981]. Participants were recruited to either a student group (which was expected to have a better level of oral hygiene) or a patient group with a total sample size of 87. This study uniquely used disclosing tablets to increase the visibility of the plaque present and recorded the amount for the full mouth by the Greene and Vermillion Index [1964] and for the anterior segment by the Stain Index by Shaw and Murray [1977]. The stain index involved drawing an outline of the disclosed plaque on an enlarged picture of the relevant tooth with 4mm grid squares. The area of plaque was then estimated by the area of the total tooth surface which was disclosed. For irregularity the Sandalli Index [1973] was used which is also a categorical measure of crowding as opposed to quantitative measure. They supported the finding of increased plaque accumulation around mal-aligned teeth and suggest that the area measure of plaque accumulation may be more sensitive in detecting small differences thereby a correlation would be easier to detect. They also note, however, that the position of the segment of teeth may be more important in that mandibular teeth tended to have increased plaque scores when compared to maxillary teeth regardless of the degree of mal-alignment.

The final study to discuss is the only longitudinal cohort study which was reported by Davies and colleagues in 1991. It forms part of a larger cohort carried out in South Wales and a sample of 417 participants from the original cohort of 1015, all who were deemed at baseline to have significant occlusal conditions that warranted orthodontic treatment, were followed up after 3 years from baseline examination [Davies TM *et al*, 1991].

This longitudinal study allowed investigation of the changing level of plaque within subjects with time and also the differences seen between those who did and did not receive orthodontic treatment. Plaque was again measured using the Silness and Löe Plaque Index [1964] and anterior crowding was defined as severe if one or more of the 12 anterior teeth were displaced from the arch by 2mm or greater.

They found significant differences between the plaque levels of both groups at baseline and found that oral hygiene was significantly better in those that subsequently went on to receive orthodontic treatment. The difference in plaque level was wholly explained by a difference in the frequency of tooth brushing at baseline as opposed to any socio-economic factors.

The 3 year follow-up data also showed a significant difference between the groups, although plaque levels had reduced in both over the 3 years possibly owing to improved awareness and motivation. When background factors were controlled for this time, however, the treated group still had consistently lower plaque scores. This was strongly related to the subject's baseline plaque score and was found in both the anterior and posterior regions of the mouth.

Of the initial sample, 113 were recorded as having severe crowding at baseline. 38 received orthodontic treatment whilst 75 did not. These two groups were analysed separately to see if the plaque scores altered following orthodontic treatment. They found that the treated group had significantly lower levels of buccal plaque around anterior and posterior teeth. Whilst this seems to confirm a relationship between incisor irregularity and plaque accumulation the authors were careful to highlight that levels improved in the posterior teeth as well which generally show less mal-alignment. As the irregularity of posterior teeth would not have altered significantly with orthodontic treatment it is possible that the effects seen are a result of a behavioural improvement in those receiving orthodontic treatment. It may be that irregular teeth pose a 'modest disadvantage' but the improvement in oral hygiene may be due to the regular attendance at orthodontic appointments and oral hygiene instruction.

A brief review of the literature has shown that there are still questions as to the role of irregular teeth in plaque accumulation and oral hygiene efficacy. This is probably due to the number of confounding factors between experimental and control groups and the lack of sensitive measures for both parameters. This DDSc project hopes to elicit a clearer idea of the complex relationship between crowding and plaque by utilising a sensitive measure of mature plaque and by following the same group of patients through from baseline to alignment.

The idea for this research project came about from the development of a sensitive measure of plaque deposits in vivo and it's potential to overcome at least some of the problems encountered in previous studies. This study may elucidate if there is a relationship between the degree of tooth irregularity and plaque deposits which, in turn, may lead to a health gain after orthodontic treatment in cases with severe crowding.

5.0 AIMS AND OBJECTIVES

5.1 Aim

To determine the longitudinal relationship of anterior tooth alignment and intra-oral plaque accumulation during orthodontic fixed appliance treatment.

To determine plaque accumulation and distribution using novel intra-oral imaging techniques.

5.2 Objectives

1. To quantify plaque accumulation on the labial surfaces of anterior teeth by visualising red fluorescence using the Toothcare™ device.

2. To test the reliability of a chair side scoring system of plaque quantity utilising the Toothcare™ light.

3. To compare the sensitivity of the Toothcare™ light to a digital camera with the ability to record a similar image displaying red fluorescent plaque (QLF-D Biluminator™).

4. To compare the reliability of measuring Little's Irregularity index by hand with dial callipers compared to the use of a Reflex Metrograph.

5. To correlate the degree of crowding measured in the labial segments to the amount of plaque detected on the labial surfaces of the teeth.

6. METHODS AND MATERIALS

6.1 Design

This study was a prospective observational cohort study looking at a cohort of adolescent patients attending for fixed Orthodontic appliance therapy. As this was an observational, non-intervention, study there was no randomisation of participants. The longitudinal nature of the study allows patients to act as their own controls with the main variant being time.

6.2 Subjects and inclusion / exclusion criteria

Consecutive patients attending the Countess of Chester orthodontic department for the start of treatment were invited to participate in the study. A cohort of 13 patients (9 females and 4 males) were subsequently recruited who met the following inclusion and exclusion criteria:

6.2.1 Inclusion Criteria

Subjects recruited to the study had the following inclusion criteria:

1. All subjects were consented to take part in the study.
2. They were all in the permanent dentition.
3. They were recruited consecutively prior to the placement of fixed appliances.

4. Treatment involved upper and lower pre-adjusted edgewise appliances, with all subjects receiving MBT prescription straight wire appliance orthodontic brackets.

6.2.2 Exclusion criteria

1. Patients with disabilities that may affect manual dexterity and oral hygiene practices.
2. Cleft-lip +/- palate patients who again may have other variables impacting on oral hygiene.
3. Patients receiving treatment with any other appliance prescription to standardise the effect of bracket profile on oral hygiene. A straight wire appliance system was also chosen (as opposed to the tip-edge system) as the aligning and levelling phase of treatment is completed early in treatment.

All subjects agreed to participate in the study following implementation of fully informed and written consent, as required by the Local Research Ethics Committee. The mean age of patients recruited was 13.4 years with a range of 11.9 – 17 years. All patients received treatment from the same clinician (AM).

6.3 Setting

Clinical data was collected from patients attending the Countess of Chester Orthodontic Department. Analysis of the data, including use of the reflex metrograph, was carried out at Liverpool University Dental Hospital.

6.4 Ethical Approval

Ethical approval was sought from the Liverpool Adult Research Ethics Committee as this clinical trial involved adolescent patients and required a minor intervention for data collection (extending treatment times) although no intervention altered the patient's course of treatment. A protocol and sample information and consent forms were submitted to the Ethics Committee in September 2009 and approval was granted in October 2009 (REF: 09/H1005/64).

Following the development of a new camera to record images of the plaque fluorescence an amendment to the ethical approval was granted for a sub-set of patients to determine the reproducibility of the Toothcare device in recording plaque deposits. Examples of the patient information leaflets and consent forms used are shown in Appendix A.

The project was also registered with the Royal Liverpool and Broadgreen University Hospital Trust Research, Development and Innovation department (REF: 3894).

6.5 Methods

6.5.1 Recruitment and Anonymisation of Data

Consecutive patients attending the Countess of Chester Orthodontic department prior to fixed appliance therapy were invited to take part in the study. Written information sheets and consent forms were given to the patients and, in the case of those under 16 years of age at the start of treatment, further consent was obtained from the parent / guardian attending with them.

Following recruitment to the study each subject was assigned a study number which was used in all subsequent recordings of plaque and crowding data to anonymise the samples. This study number was recorded along with the patient details on a log-sheet which only the primary investigator had access to. Personal details were never used in conjunction with the data collection and analyses, instead only using the unique study identifier records were anonymised. The participant number was then used to allow the ability to unify all the data for individual patients without the need for use of personal information.

6.5.2. Timing of Samples

Clinical recordings of plaque accumulation on the 12 anterior mandibular and maxillary teeth and sectional alginate impressions of these areas were collected at various time points. T1 was recorded immediately prior to placement of the fixed appliance, before any brackets were bonded to the teeth. Recordings were then taken at each subsequent orthodontic appointment to adjust the appliance, usually with an interval of 6-8weeks, until the final recording was taken when it was possible to place a 0.019” x 0.025” stainless steel archwire with a maximum follow-up of 1 year and a minimum follow-up of 6 months.

6.5.3 Plaque data collection

A record of the degree and site of plaque accumulation would be recorded at the start of each visit for data collection. The fluorescent plaque deposits were highlighted by using the hand-held Toothcare^{TM} device which is produced by Inspektor Research Systems in Amsterdam.

The handheld Toothcare^{TM} light was used to complete a plaque chart (Appendix B), before any adjustments were made to the appliance. This avoided disturbing any plaque deposits that were present to prevent artificially altering the distribution and quantity of plaque on the anterior teeth. The participant study number was recorded on the top left corner of the plaque chart to allow it to be compared to the crowding record sheets at a later date. The plaque charts were kept in each participant's clinical notes until they reached the end-point of data collection, but contained no identifying data about the patient apart from the study number. Shown below is a diagram representing the data collection sheets illustrating that each tooth was divided into sections: mesial, distal, incisal and gingival.

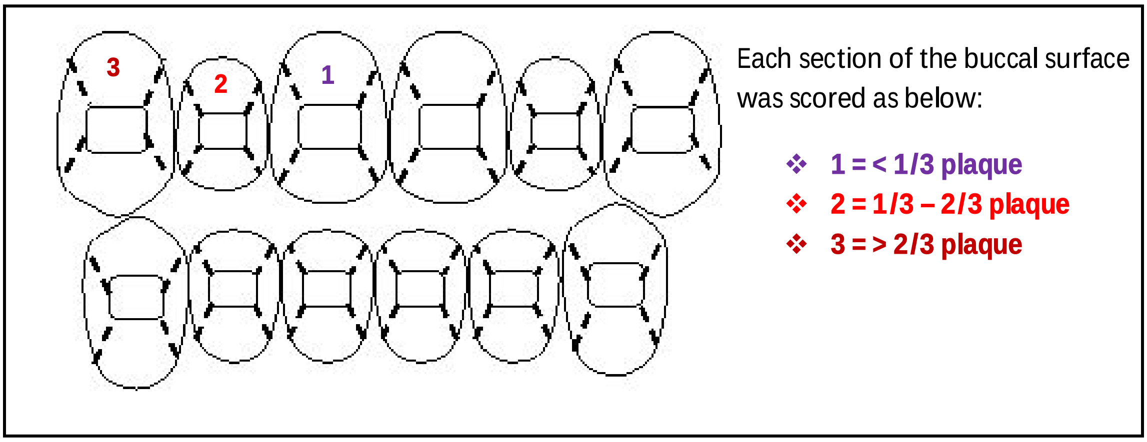

Figure 6.1: Example of chart used to record the site and extent of plaque desposits observed clinically.

Using the Toothcare™ light to detect fluorescent plaque, each section of the anterior teeth was given a score based on the Quigley Hein Index [1962]. An ordinal scale from 0-3 was used where 0 meant no plaque was present up to a maximum score of 3 where greater than 2/3 of the surface displayed the presence of fluorescent plaque. Similar to previous plaque indices, this was not a continuous scale, however, it did allow some ranking of the degree of plaque accumulation rather than recording dichotomous data of present or not. Below is an example of the image seen using the Toothcare™ light. The lower left central incisor would score a 3 on the mesial aspect and the lower right central incisor a 2 on the mesial aspect.

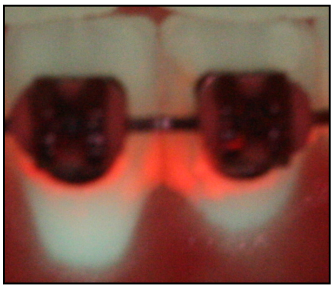

Figure 6.2: Image taken using the QLF-D Biluminator to illustrate the fluorescence seen with a Toothcare light (which does not have a mechanism to record an image)

<u>Repeatability of Plaque Scoring method</u>

To assess the intra-observer repeatability of the plaque scoring method described above measurements were repeated and the two records compared. As the Toothcare™ does not record an image, the two readings required to be taken at the same visit for consistency in the plaque deposits being scored. Two immediately consecutive measurements, however, would not be a robust measurement of repeatability as it would be possible for the observer to be influenced by memory of the first recording. Therefore, a reading was taken at the start of a visit and then after half an hour (the length of an appointment to adjust the appliance). This time delay would help to avoid bias in the repeat measures and to further avoid bias the measurements were recorded on separate charts and the scores later entered into a database to compare first and second recordings. As the data recorded was ordinal data, the intra-observer reliability was evaluated with kappa statistics. This would compare the degree of exact concurrence of scoring and any differences, even if only one group apart, would indicate disagreement.

<u>Reproducibility of Plaque Scoring</u>

After commencement of the study a digital camera was developed which could record the image seen using the Toothcare™ device. This allowed a comparison of the intra-observer reliability over time by measuring the plaque deposits when the subject initially attended the clinic using the Toothcare™ light and subsequently, after a delay of a week, the digital image was similarly scored. The separate recordings were again compared for relative agreement using kappa statistics looking at exact consensus between recordings. The QLF-D Biluminator™ is similarly manufactured by Inspektor Research Systems in Amsterdam.

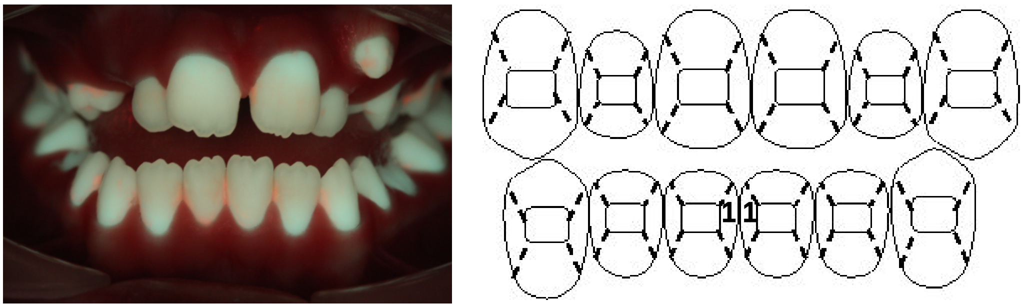

Figure 6.3: Example of image obtained with QLF-D Biluminator which could be compared to a plaque score measured at the chair side indicating that the mesial aspect of the lower central incisors would both be scored a 1

6.5.4 Crowding data collection

For each subject that entered the study, at each of the time points, sectional alginate impressions were taken of the maxillary and mandibular anterior teeth (12 in total). These impressions were taken after the plaque chart had been completed to ensure no disturbance to the plaque deposits present on the teeth prior to recording its distribution and amount. The archwires were removed to allow an impression of the full buccal surface of these teeth. The alginate impressions were then disinfected and plaster models were poured with the patient's study participant number as identification marked on each cast.

Little's Index Scoring with Dial Callipers

Each sectional model was measured to determine the irregularity of the labial segment teeth. The Little's Irregularity Index [Little, 1975] was chosen to measure the crowding as it is representative of the degree of tooth irregularity rather than a tooth size / arch length discrepancy and it was thought this may be more relevant in terms of plaque stagnation sites. Each model was measured in Liverpool Dental Hospital, without reference to the patient's plaque chart (which was in their clinical notes in the Countess of Chester). This aimed to avoid bias of the measurements through knowledge of the patient's previous plaque score. There was at least 2 weeks delay between completion of the plaque chart at the clinical appointment and measurement of the plaster models in order to avoid recall of the degree of plaque accumulation for each model being scored. The measurements were recorded on a 'Little's score record sheet' (Appendix C).

Little's Index Scoring with Reflex Metrograph

All models were re-scored using the Reflex Metrograph (manufactured by Ross Instruments, UK). Each model was mounted on the platform in front of the semi-silvered mirror and the light marker moved directly over the contact point of interest. Using this light marker avoids contacting or marking the cast (which is important to avoid fiducial marks which may bias repeat measurements to test reliability). Computer software is used to determine the co-ordinates of each point and the distance between the points is calculated. The use of a computer may reduce the measurement error involved in reading the distance on a dial calliper but does not avoid the error of landmark identification. Reported accuracy of values recorded are $\pm$ 0.2-0.3mm [Speculand et al, 1988]. One possible disadvantage of this 3-dimensional method is that it measures discrepancies in the vertical plane as well, whilst Little's traditionally only looks at horizontal distances. This may lead to a systematic over-estimation of the irregularity when using the reflex metrograph.

The study models were secured on an inclined block on the recording table of the reflex metrograph. Background light was eliminated and an angle-poise lamp used to direct light onto the sectional model. The 0.3mm light spot of the reflex metrograph was then positioned over each contact point using the reflected image and the accuracy of the superimposition was checked by viewing the model in different planes (thus minimising parallax errors). Once the position was confirmed the co-ordinates were recorded by pressing the foot pedal and this was repeated for each contact point on each model.

Once the readings for one model were complete, linear distances were calculated by the computer for adjacent contact points. These readings were then recording on the Little's scoring sheet for that study participant. The same process was repeated for each model collected.

Reliability of Little's Index scoring and comparison of accuracy of methods

A random selection of 8 models were re-measured using both methods after a period of at least 2 weeks. The models were selected using a random number table to determine which participant and which sample would be re-measured. The first and second readings were then compared to determine intra-observer reliability for each method (hand scoring with the dial callipers and scoring via the reflex metrograph) using Dahlberg's formula of method error [Dahlberg G, 1940] :

$$\text{Method Error} = \frac{\sqrt{\sum d^2}}{2n}$$

d = difference between first and second measurement

n = number of sets of measurements

This estimates the random error of the measurements and the Wilcoxon signed ranks test assesses the level of significance of the systematic error of the measurements.

To compare the digital and hand scoring methods, the difference in mean scores was also compared using the Wilcoxon signed ranks test.

6.6 Statistical Analysis

The reliability of both plaque scoring and Little's index measurements were determined by carrying out repeated measurements. Conditions to be fulfilled for a repeatability test are:

- The same measurement procedure
- The same observer
- The same measuring instrument
- The same location
- Repetition over a short period of time

As the Little's index measurement data was continuous, the Dahlberg formula was used to determine the magnitude of difference between repeated measurements. To determine if there was a systematic difference between each set of measurements the repeated measures were then assessed using a Wilcoxon signed ranks test. This non-parametric significance test was chosen as the primary data was not normally distributed and it can be used to compare two related samples or repeated measurements.

Non-parametric statistics are useful for analysing non-normally distributed data as they make fewer assumptions about the data than parametric tests. They can also be used when the scale used is considered weaker than that required for a parametric procedure: such as rank or ordinal data similar to the plaque score used in this study. The disadvantage of non-parametric procedures, however, is that they are less powerful than parametric tests.

The ordinal plaque data was tested for repeatability by multiple recordings using the Toothcare™ light. To test intra-observer agreement Cohen's kappa statistic was used to determine the proportional exact agreement between each reading. A score of 1 implies perfect agreement whilst $\kappa = 0$ suggests that agreement is no better than that which would occur by chance. There are no objective criteria for judging intermediate values, however, the degree of agreement may be determined as [Petrie and Sabin, 2009]

- Poor if $\kappa < 0.00$
- Slight if $0.00 \leq \kappa \leq 0.20$
- Fair if $0.21 \leq \kappa \leq 0.40$
- Moderate if $0.41 \leq \kappa \leq 0.60$
- Substantial if $0.60 \leq \kappa \leq 0.80$
- Almost perfect if $\kappa > 0.80$

Similarly Cohen's Kappa statistic was used to compare the sensitivity of plaque measurement when using the Toothcare™ device at the chair side compared to scoring the plaque distribution from a recorded digital image from the QLF-D Biluminator™.

Good intra-observer agreement would suggest that each form of measurement was comparably sensitive in detecting mature plaque deposits.

Following examination of the reliability of the measurements used, the plaque and Little's data was examined over time. As the primary data was not normally distributed for either measurement and the plaque scores consisted of ordinal data, median scores were used to determine the central tendency of the data. A graph of median scores over time helped to illustrate any trends that may be present but to confirm the presence of a trend a Kruskal-Wallis test was used to compare the scores at different time points. This test is a distribution-free alternative to the parametric analysis of variance test (ANOVA). The evidence of a statistically significant difference between time points would help to confirm a trend over time. To determine where the differences lay, the Wilcoxon signed ranks sum test was used to compare T1-T2, T2-T3 and T3-T4.

To determine if there was a correlation between then degree of crowding and the plaque accumulation amongst participants, the non-parametric Spearman Rank Correlation Coefficient was calculated. This is based on the assumption that:

1. Data consist of a random sample of *n* pairs of numeric or non-numeric observations.
2. Each pair of observations represents two measurements taken on the same object or individual, called the *unit of association*.

The Bonferroni method was also applied to the correlation statistics as multiple comparisons were carried out on the same data. The more statistical tests that are carried out on a sample of data, the increased risk of a type I error. To adjust for this the alpha level must be set lower (determined by α/k: where k is the number of statistical tests completed) or the p value is multiplied by the number of statistical tests to determine a more realistic p value (kP). [Bland and Altman, 1995]

7. RESULTS

7.1 Description of subjects

13 patients consented to be part of the study prior to having fixed appliances placed. There were 9 females and 4 males, this distribution reflects the general trend for increased numbers of females pursuing orthodontic treatment than males. The mean number of appointments that each participant was observed for was 4 visits.

7.2 Distribution of sample

As the sample size was small (less than 30 patients recruited) the data was not normally distributed and, therefore, non-parametric tests were chosen to compare groups of data (Appendix D).

7.3 Reliability of Little's Irregularity Index – Hand scoring

As described in the methods chapter, a random sample of 8 models were re-measured using the dial callipers after at least 2 weeks. A comparison of the difference between each measurement of contact point displacement was carried out using Dahlberg's formula of method error (Appendix E).

This gave a method error value of ***0.17mm***.

The paired observations were then assessed using a Wilcoxon signed ranks test which is a non-parametric significance test of 2 related samples.

The resulting p-value is shown below:

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The median of differences between Hand1 and Hand2 equals 0.	Related-Samples Wilcoxon Signed Rank Test	.513	Retain the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

Table 7.1: Wilcoxon signed rank test of 1st and 2nd Little's hand scores with p>0.05 suggesting no statistically significant difference between the paired measurements

This meant the null hypothesis was retained and this suggested no statistically significant difference between the measurements taken on both occasions.

7.4 Reliability of Little's Irregularity Index – Reflex Metrograph

A similar test of reliability was carried out for the measurements taken with the reflex metrograph. Again a random sample of 8 models were re-measured after a period of 2 weeks. The resulting difference between both measurements was assessed using Dahlberg's formula.

The resulting method error value = ***0.22mm***.

Comparison of both readings by the Wilcoxon signed ranks test found the following p-value:

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The median of differences between Reflex1 and Reflex2 equals 0.	Related-Samples Wilcoxon Signed Rank Test	.213	Retain the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

Table 7.2: Wilcoxon Signed Rank Test of 1st and 2nd Reflex Metrograph readings with p>0.05 suggesting no statistically significant difference between paired readings

Again the null hypothesis is accepted and there is no statistically significant difference between the measurements taken on the two occasions.

7.5 Comparison of Hand and Reflex Metrograph scoring of Little's Irregularity Index

All sectional models collected for the study were scored both by hand using dial callipers with a scale of 0.05mm as well as being scored by the reflex metrograph. Comparison of both measurements was carried out again with a Wilcoxon signed rank test to determine if there was a difference between the two methods (Appendix G). The resulting p value = ***0.000*** which was less than the significance level set at 0.01.

This suggests that there was a systematic difference between the Little's Irregularity Index when recorded using the dial callipers compared to the reflex metrograph. Looking at a graphical representation of the differences between the measurements, using the reflex metrograph tended towards an increased score when compared with the dial callipers.

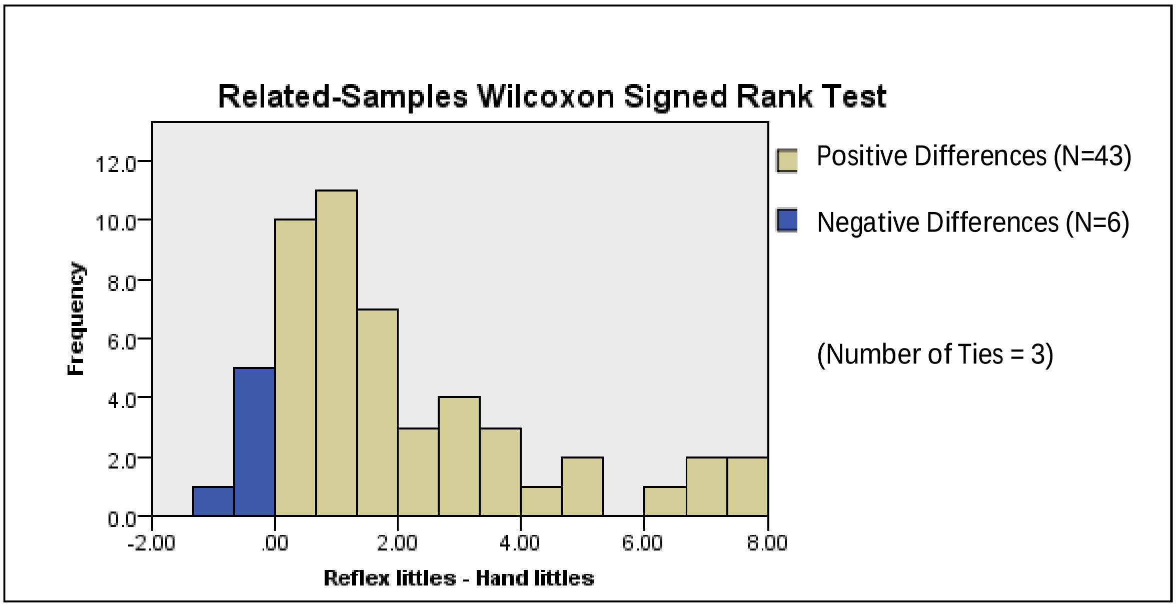

Figure 7.1: Display of positive and negative differences between Little's Index scored by the Reflex Metrograph and by hand (Reflex – Hand) showing more positive differences suggesting a tendency for the Reflex Metrograph to measure a higher score than by hand.

7.6 Reliability of Toothcare™ in recording Plaque accumulation

To compare the reliability of the scoring method employed using the Toothcare™ system, 8 patients in the study group were selected. These participants had a chart completed at the start of a treatment visit and then again at the end of the visit. The two scores were then compared using κappa statistics to determine the degree of direct agreement.

The κ value for the plaque charts = ***0.92***

This suggests good intra-observer agreement between the first and second recording [Petrie and Sabin, 2009].

7.7 Reproducibility of plaque scoring – Toothcare™ versus QLF-D™

Whilst the test of reliability suggested good intra-observer agreement, there was a very limited time delay between measurements. The development of the QLF-D camera, allowed a greater lapse of time before re-scoring to determine the sensitivity of the scoring method. Similarly 8 participants (a sub-set group of patients who had not yet received orthodontic treatment) were selected where the plaque score was recorded at the chair side using the Toothcare™ light. At the same visit digital images were recorded using the QLF-D Biluminator™ and the plaque score was re-measured by analysing these images at least 2 weeks after the initial appointment.

Comparison of the plaque scores recorded via each method was again analysed using κappa statistics (Appendix I). This time the agreement was slightly lower but would still be considered a substantial agreement [Petrie and Sabin, 2009]:

κ = ***0.76***

To determine if there was a systematic difference between the methods the Wilcoxon signed ranks test was used. The resulting ***p value*** was ***0.027*** suggesting there was a systematic difference between both readings. The graph below shows that the readings using the Toothcare™ light were consistently higher than those determined from the QLF-D Biluminator™.

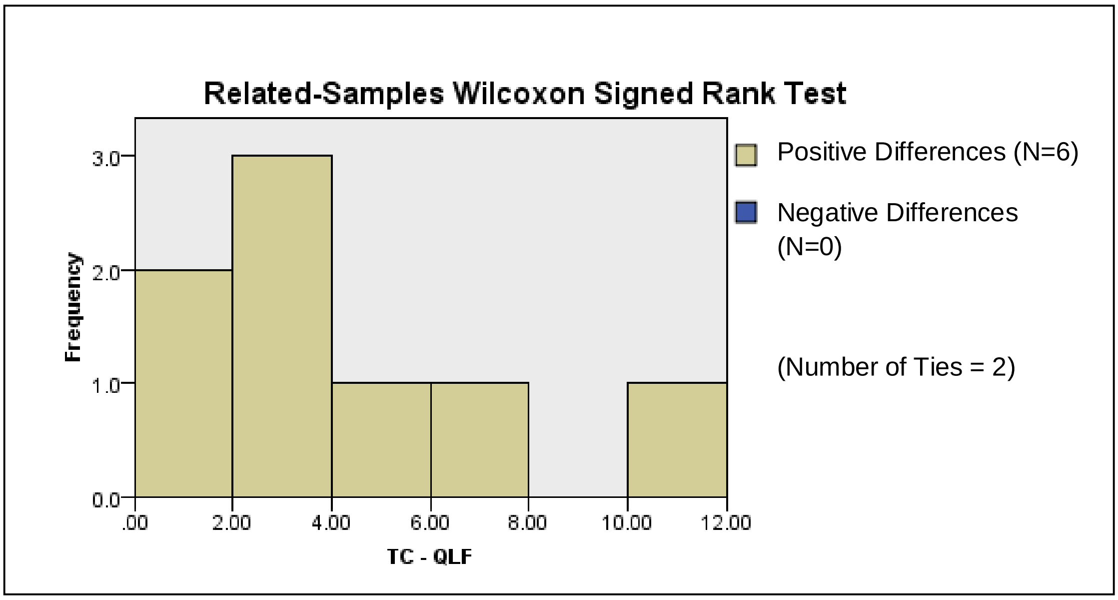

Figure 7.2: Difference between plaque scores recorded for QLF-D and Toothcare. A greater number of positive differences suggests the Toothcare recorded greater plaque deposition compared to the QLF-D

7.8 Trends of crowding and plaque deposits over time

Due to the small sample size and the ordinal plaque data non-parametric tests were considered appropriate for analysis of the data. The spread of the data for both Little's Irregularity Index and plaque score showed a skewed distribution. Below the median and spread of the data at each appointment are shown.

Plaque	1	2	3	4
Median	4	9	3	5
IQR	3	11.5	12	17
25th percentile	3	3.5	1.5	1
75th percentile	6	14	14.5	18

Table 7.3: Median values with IQR for plaque score at each visit

Little's crowding	1	2	3	4
Median	10.9	6.05	2.8	2.4
IQR	23.55	13.1	11.03	10.65
25th percentile	6.45	1.8	0.35	0.35
75th percentile	17.1	14.9	11.38	11

Table 7.4: Median values with IQR for Little's Irregularity index at each visit

If we look at the scatter plot of the medians of plaque score and irregularity score at each visit, whilst there is a definite trend for the crowding to steadily decrease over time, the plaque score is more variable. The plaque score increases at the second visit before reducing slightly again by the third visit. The plaque score appears to increase again at the fourth visit but not to such a great extent and is close to the pre-treatment plaque levels recorded.

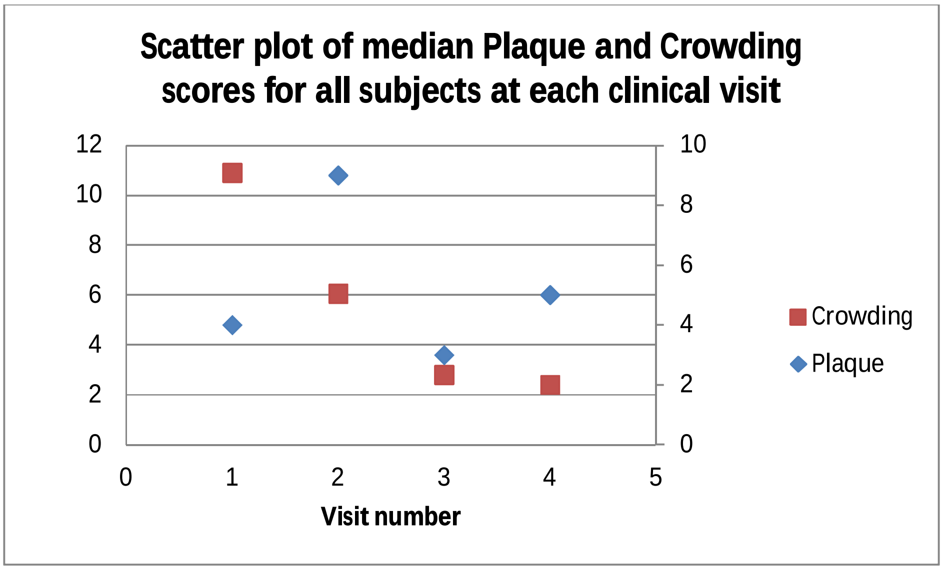

Figure 7.3 : Scatter plot displaying median plaque and Little's scores of all patients at each visit to illustrate trends in plaque deposition and degree of crowding over time.

There was considerable variability in the pattern of change in crowding and plaque accumulation over time between patients. To determine if there was a true trend, the Kruskal-Wallis non-parametric test was used to compare data from each visit. There was a statistically significant difference between the groups in terms of the Little's measurements over time $p = 0.038$. To determine where the differences were the Wilcoxon signed ranks test was then used to compare consecutive visits.

This determined that there were significant differences between the first and second and second and third visits in terms of incisor irregularity, however, there was no significant difference between the third and fourth visits. This confirms that there was an improving trend for irregularity from the first to the third visit (Appendix J).

In contrast when the trend was investigated for plaque scores over time there was no evidence from the Kruskal-Wallis test of any significant difference as $\boldsymbol{p = 0.741}$. This suggests that the trend observed in the scatter plot occurred purely by chance.

7.9 Correlation of plaque and crowding scores

The paired data of crowding and plaque deposits for each patient at each visit were compared to determine if there was any statistically significant correlation between the two factors. As the sample was small and not normally distributed, a Spearman's correlation was carried out. This is based on 2 assumptions:

- The data consist of a random sample of n pairs of numeric or non-numeric observations.
- Each pair of observations represents two measurements taken on the same object or individual, called the *unit of association*.

Correlations

			Hand littles	Plaque percent
Spearman's rho	Hand littles	Correlation Coefficient	1.000	-.375**
		Sig. (2-tailed)	.	.006
		N	52	52
	Plaque percent	Correlation Coefficient	-.375**	1.000
		Sig. (2-tailed)	.006	.
		N	52	52

**. Correlation is significant at the 0.01 level (2-tailed).

Table 7.5: The results of Spearman's correlation between plaque and crowding over the entire study suggesting a correlation coefficient of -0.375 with a p value = 0.006. Whilst this is statistically significant (p<0.05) the degree of correlation is weak.

Although the *p-value* suggests there is a statistically significant correlation between the two variables (*p=0.006 with significance set at 1%*) the strength of the correlation is weak ***r= -0.375***. We can, therefore, conclude that the quantity of fluorescent plaque is not strongly correlated with the degree of labial segment crowding. Interestingly, the negative sign suggests that the relationship is inverse with oral hygiene deteriorating as crowding improves.

To determine if the results were affected by the presence of a fixed appliance, the Spearman's correlation was repeated this time for the first visit only (no appliance present) and also for the second to fourth visits (once the appliances were in place).

The correlation at visit 1 was significant at the 5 percent level (*p = 0.03*). Again the correlation was negative ***r = -0.602***. This still suggests an inverse relationship between incisor irregularity and plaque accumulation and is stronger than the previous correlation.

Comparing the data for the second to third visits, similarly there was a negative correlation, this time not as strong as that at T1: ***r = -0.428*** but still significant at the 1 percent level (*p = 0.008*).

There is a risk with multiple statistical tests on a small sample of finding a 'significant' result when there is not one (type I error). In this case a Bonferroni correction can be applied to determine the alpha (α) significance level that should be set to determine statistical significance. This can be determined by dividing the normal significance level (0.05 or α) by the number of tests to be carried out (in this case 3 x spearman's correlation coefficient). It may, therefore, be more robust to set the α-level for these tests at the 1 percent level (0.01) in which case the correlation of plaque and crowding at the first visit of ***r = -0.602*** would not be statistically significant.

Whilst most patients showed a consistent decrease in anterior crowding over time the trend for plaque accumulation was much more variable and descriptive data showing individual changes over time are included in Appendix K.

8. DISCUSSION

8.1 Summary of main findings

1. Plaque accumulation on the labial surface of the anterior teeth does not appear to be strongly positively correlated with the degree of irregularity of these teeth.
2. Whilst there was a consistent trend of reduced dental irregularity over time the pattern of plaque accumulation did not show a consistent trend and was much more variable.
3. An ordinal plaque scoring index used in conjunction with the Toothcare™ light shows acceptable intra-examiner repeatability and is a useful measure of plaque deposits amongst orthodontic patients.
4. Reasonable reproducibility has been shown between a chair side plaque index and measurement from a digital QLF-D Biluminator™ image, although the 2-dimensional image tended to exhibit lower scores.
5. Hand measurement of Little's Irregularity Index can be carried out with reasonable accuracy and intra-observer consistency.
6. The reflex metrograph showed slightly poorer accuracy in measuring Little's Irregularity Index in this study.
7. Using the reflex metrograph to measure linear distances for Little's Irregularity Index consistently over-estimates the degree of crowding when compared to the use of a dial calliper. A more complex programme taking into account measurements in the horizontal plane only (perhaps measuring relative

displacement from a pre-defined ideal arch) may be preferable to improve accuracy with this method.

8.1.1 Poor correlation between degree of crowding and quantity of plaque amongst orthodontic patients

The standard of oral hygiene recorded amongst patients is extremely variable and plaque accumulation is affected by multiple factors. Although literature reviews researching the relationship between poorly aligned teeth and the presence of plaque and periodontal disease suggest that there may be a correlation there are a lot of contradictory results in the primary literature [van Gastel, 2007; Deidrich 2000].

The great diversity in study method may account for the conflicting information, however, those studies which have demonstrated correlations between the degree of tooth irregularity and plaque accumulation often report very weak associations. The cross-sectional study by Buckley in 1981 suggested that plaque levels scored using the Silness and Loe Index [1964] showed a positive correlation with the degree of dental mal-alignment (measured using the van Kirk and Pennel Index, 1959) but the Pearson Correlation value was 0.14. This suggests that whilst crowding may play a role in plaque accumulation, it is not the main aetiological factor and the relationship is weak [Buckley, 1981] with only about 2% of the association attributable to crowding.

Griffiths and Addy in 1981 found a slightly greater correlation coefficient in their cross-sectional study of 87 students and dental patients. They used two methods of measuring the quantity of plaque present: firstly they used the Greene and Vermillion Index for all teeth present excluding third molars [Greene and Vermillion, 1960] scoring per 1/3 coverage of the tooth surface. Then they used the stain index reported by Shaw and Murray [1977] to score the anterior teeth. The stain index uses a magnified chart of tooth surface divided into 4mm squares and the observer shades the area of disclosed plaque seen before counting the squares in the shaded area. This method was thought to be able to better detect small changes in plaque present. The correlation of irregularity of the anterior teeth and the stain index was 0.31 which is slightly higher than other studies reported. The authors suggest this is due to the method they used to score the presence of plaque. In a sub-set of patients with good oral hygiene, the correlation for the whole mouth plaque score and contact point irregularity was slightly greater r=0.62. This increased r value may be due to the plaque index they used [Green and Vermillion, 1960] which quantified plaque across the whole of the tooth surface, rather than the gingival margin only as occurs with the Silness and Loe Index [1964]. Similarly the cross-sectional data from the Cardiff cohort study was analysed by Addy et al in 1988 and they suggested a modest but statistically significant positive correlation between mean plaque and irregularity indices. Again, however, the correlation coefficient was 0.24 and based on an r^2 value 0.058, about 6% of quantity of plaque seen could be explained by the irregularity of the teeth.

In 2006, Ngom and colleagues studied the strength of the relationship in the mandibular and maxillary anterior teeth separately. They restricted observations to the anterior teeth as they felt this would avoid any effects of altered manual dexterity amongst patients. Dividing the analysis into maxillary and mandibular teeth was also suggested as in the previous study by Griffiths and colleagues, the mandibular teeth tended to increased plaque accumulation which they felt over-powered the effect of tooth irregularity

[Griffiths *et al*, 1981]. Despite separation of maxillary and mandibular teeth, the correlation coefficients were 0.225 and 0.338 respectively and indicate a moderate relationship only.

Therefore, although some reviews may suggest an association between crowding and plaque accumulation the strength and degree of the association may be very small. In the case of this project the sample size was very small and this may mean the sample was underpowered. This combined with the use of non-parametric statistics which also tend to be underpowered (as they make fewer assumptions about the data than parametric statistics) can lead to the risk of type 2 errors of analysis. This means that if a relationship is present, there was not adequate statistical power to definitively find it. Having a larger sample may have improved the robustness of the statistical analysis and this combined with the longitudinal study design would have helped to determine if there was a causal relationship as cross-sectional investigations are limited by only inferring association but not determining direct causality.

The longitudinal design of the study did demonstrate the very varied nature of the pattern of plaque accumulation within patients and this may be another reason why a strong association was not found. Large cross-sectional studies may have found correlations by chance but this longitudinal study demonstrated that plaque levels can alter considerably even in the absence of change in incisor irregularity or that plaque levels remain constant despite large alterations of dental alignment.

Other factors which may have impacted on the level of oral hygiene and, therefore, masked any relationship which exists between plaque and crowding include the presence of a fixed appliance, behavioural factors including level of oral hygiene at the start of treatment and the effect of continuous reinforcement of oral hygiene instruction at each clinical appointment. In addition to this the patients were all consented and, therefore, aware they were part of a study looking at plaque accumulation and being conscious of the close monitoring of their oral hygiene this may have affected their normal oral hygiene practice and behaviour. This phenomenon has been reported in other clinical experiments and is known as the Hawthorne effect [Franke and Kaul, 1978]. Unfortunately it would not be possible to blind the patient to the use of the Toothcare™ light and full informed consent is of paramount importance, therefore, this bias could not be eliminated.

On first observation the median plaque scores appear to increase after the first visit suggesting oral hygiene deteriorated following the placement of the fixed appliance. This is to be expected, but as the initial aligning wires were in place, this effect may have over-powered the effect of initial reduction in irregularity. This may in part explain the negative Spearman's correlation observed as the visit in which the greatest improvement in alignment was generally seen was also the first visit the patient was adapting to the fixed appliance. Whilst a longitudinal design is still preferable, it may have been useful to bond the appliance and leave it passive for a visit to separate the effect of the fixed appliance and tooth alignment. This, however, would have increased the overall treatment time and is not ethical in terms of the increased risk to the dentition.

The Kruskal-Wallis test, however, demonstrated that there did not appear to be a strong trend in the pattern of plaque accumulation despite the observed trend for crowding to improve over time. This may in part be due to the small sample size being unable to detect any difference from one visit to another and would also suggest that there is not a simple relationship between crowding and plaque.

As this study was to determine the effect of alignment, participants were followed until they reached a 0.019” x 0.025” stainless steel archwire and the end of the levelling and aligning phase. It would have been beneficial to continue to follow this cohort until after appliances were debonded and during the retention phase to determine if removing the fixed appliance impacted on the level of oral hygiene and to compare the baseline and post-treatment plaque scores to determine if there was any change following orthodontic treatment. It is still difficult, however, to determine the extent to which any changes may be attributed to improved dental alignment as the regular reinforcement of oral hygiene instruction at orthodontic appointments may bring about a behaviour change. This has been suggested as an explanation for improved oral hygiene observed in a cohort of orthodontically treated patients compared to a similar group that had not received orthodontic treatment [Davies *et al*, 1991]. The authors of this study felt that behavioural factors such as the number or times a day a patient brushed their teeth was more important than the degree of crowding present. In addition, this study was a cross-sectional study and did not account for selection bias in those patients selected for orthodontic treatment compared to those that were not. Before commencing orthodontic treatment, patients are screened regarding their oral hygiene and, therefore, the patients in the treated and untreated group would have been systematically different from baseline.

Some research has suggested that different extremes of oral hygiene compliance may alter the relationship observed between plaque levels and crowding. All participants in this study were recruited from the Orthodontic Department at the Countess of Chester. The protocol in the department is that most patients are referred for Dental Health Education advice before commencing treatment and this is generally over a period of three visits. Before active orthodontic treatment is initiated the average full mouth plaque score should be less than 15% according to the O'Leary Plaque Index [1972]. This implies that the majority of patients recruited should have had good oral hygiene and low plaque scores at the start of the study.

Authors such as Ainamo and Behlfelt believed that correlation was more obvious in those patients with good oral hygiene, whilst those with poor oral hygiene tended to mask any association [Ainamo, 1972; Behlfelt 1981]. This was also found in the investigation by Griffiths and colleagues where an association between the Greene and Vermillion Index [1960] and contact point displacement was only strongly correlated amongst a sub-group of patients with good oral hygiene. This would tend to suggest that this study would be more likely to find a correlation, however, other researchers have suggested the opposite is true. The cross-sectional study by Ashley and colleagues [1998] felt that they failed to find a correlation as the participants involved had generally good oral hygiene. They felt that with very small plaque levels, any change would be difficult to detect and that the relationship would be more obvious in those patients with more moderate oral hygiene practices [Ashley et al, 1998]. This may also explain why a study of student dentists and nurses also failed to find any association [Ingervall, 1977] as they would have been more aware of good oral hygiene behaviours. This may be explained by the theory suggested in another periodontal investigation [Glans et al, 2003] where patients with a crowded dentition had received more oral hygiene instruction prior to orthodontic treatment. This study of 97 subjects found that the baseline periodontal variables were the same regardless of whether they were in the severe crowding group or the no crowding group. After the teeth were aligned, gingival health was improved to a greater extent in the group which started with severe crowding. This may be because they were more meticulous when brushing their teeth before treatment, as they were aware of the difficulty around the irregular teeth, that when crowding was resolved the conditions for cleaning were enhanced even more and there was an even further improvement in oral hygiene. This may also partly explain the negative correlation seen in this study as those patients with a crowded dentition may have had more oral hygiene instruction before treatment and this may have led to improved plaque scores when compared to those with better dental alignment.

Therefore, patients with good oral hygiene behaviours at the start of treatment may not show a great change in plaque accumulation despite improved alignment of the teeth. In addition to this, those patients with more severe crowding may have been given a greater amount of oral hygiene instruction prior to treatment and, therefore, started with a lower plaque score than those with more average incisor alignment.

Both of these factors may explain why the baseline correlation suggests an inverse relationship between plaque and crowding; orthodontic patients at the Countess of Chester generally have low plaque scores before treatment is initiated; those with particularly crowded dentitions may have benefitted from more frequent oral hygiene instruction than others.

8.1.2 Good test re-test repeatability of plaque scoring system

The high κ score for repeated plaque scoring with the Toothcare™ light suggests that this method of quantifying plaque is reasonably reliable. It has an advantage over the most commonly used Silness and Loe plaque index [1964] as it assesses the whole tooth surface, not just the gingival area. Whilst gingival plaque may be more relevant for periodontal disease, as previously discussed, the aetiology of periodontal disease concerns the presence of specific species of bacteria and the host inflammatory response rather than the absolute amount of plaque present. Plaque presence across the tooth surface has an important role in enamel demineralisation, especially in those patients undergoing fixed appliance treatment. Another advantage of this system is that the plaque level can be scored multiple times in one visit which is not possible with the Silness and Loe Index [1964] which requires removal of the plaque debris with a probe.

The Toothcare™ light is especially useful as it highlights areas of plaque accumulation much more easily than if examining the tooth surface using white light alone [Thomas, 2010]. One concern, however, may be that not all plaque present will fluoresce and, therefore, the total quantity of plaque may be underestimated in comparison with the use of disclosing tablets [van der Veen *et al*, 2006]. Since red fluorescent plaque, however, is indicative of more mature and potentially more harmful bacteria implicated in oral disease the quantities detected using this light may be more relevant to extrapolate to risk of developing disease compared with less discriminatory methods.

The creation of the QLF-D Biluminator™ system is an exciting development which may make the method of measuring plaque levels even more robust as images may be saved and archived for future analysis and assessed by multiple examiners over longer periods of time to examine intra and inter-examiner reliability. In addition to this it may be possible to quantify the area of plaque covering the tooth surface more precisely using the planimetric method as described by Pretty and colleagues [2005]. This would enable detection of small changes in plaque score and much more precise measurements than the subjective estimations made at the chair side. This would also show where plaque tended to accumulate and allow patients to be educated about this.

Whilst this study showed acceptable reproducibility between the plaque scores obtained with the Toothcare^{TM} light and the QLF-D Biluminator^{TM} there was also a tendency for the digital camera to detect less plaque than the hand-held light. Part of this may be the greater degree of sensitivity to the method of using the digital camera and lack of expertise of the operator. Another explanation for the difference, however, is that the use of the light at the chair side allows analysis of the 3-dimensional surface of the teeth. The digital image is a 2-dimensional representation and, therefore, it is possible that some areas of the tooth surface may be missed and the actual surface area of the tooth with debris may be underestimated. Another study carried out in Brazil compared a camera which could detect red auto-fluorescence with a two-tone disclosing solution. This study found good reliability and discriminatory power in the quantification of plaque using the fluorescent camera and a good correlation between disclosing and fluorescent methods although the disclosing agent tended to show a higher area of mature plaque than the camera. [Raggio *et al*, 2010] This is similar to the results of this study, where the camera appears to have under-estimated the degree of plaque accumulation compared to the Toothcare^{TM} light.

8.1.3 Poorer repeatability of Little's Irregularity Index with Reflex Metrograph

Although it was anticipated that measurement using a small pointer and use of a computer programme to calculate precise distances (removing the bias of observer error) should be more reliable, the accuracy of scoring using the reflex metrograph was found to be less than the hand scoring. This can be explained by the added difficulty of assessing points in 3-dimensions with the reflex metrograph, thus repeated errors may occur as the pointer requires to be positioned precisely vertically as well as antero-posteriorly and transversely on the contact point. The hand scoring method is carried out with the callipers parallel to the occlusal plane, therefore, there is no requirement to position the tips vertically in the contact embrasure. [Little, 1975] Using the reflex metrograph the light spot should be placed directly over the perceived anatomical contact point of the tooth which will introduce errors in the vertical dimension as well. Precisely identifying a point on a 3-dimensional model repeatedly can be difficult and may be improved by marking the models with fiducial marks, however, this would introduce a systematic bias into the measurement.

Comparison of the paired measurements carried out using vernier callipers and the reflex metrograph indicated a systematic difference between both measurements. The reflex metrograph technique tended to record a larger contact point displacement distance than the hand scoring method. One explanation for this could be the introduction of a vertical displacement between contact points measured as discussed in the previous section [Almasoud *et al*, 2010]. Another possibility is that some participants had slight spacing between the teeth and this would be recorded as increased distance but would not represent an increased irregularity. It may be possible to overcome this effect by using a method whereby an ideal archform is created as a reference plane to which the contact point displacements are measured at right angles. A positive score could be attributed to labial displacements and negative to lingual displacements with the archform itself being the zero plane. The relative distances of each contact point to the reference plane could be subtracted to give a total contact point displacement.

8.2 Study Limitations

8.2.1 Sample

Sample Size

Unfortunately the sample size for this study was quite small owing to the need to recruit patients prior to bonding of the fixed appliance for baseline recordings and the delay in initiating the study whilst awaiting ethical approval. Whilst a larger sample would reduce sampling error and reduce the chance of type 2 statistical errors, the rigorous study methods employed aimed to reduce this effect.

Site and participant selection

All patients were recruited from the Orthodontic department at the Countess of Chester Hospital and this may introduce systematic bias when extrapolating findings to other populations. Patients accepted for treatment generally have good oral hygiene and this may have reduced our ability to detect a correlation between plaque and crowding. The small sample may also have prevented the possibility of seeing a wide variation in crowding and plaque levels at baseline. Also, as these patients were part of a second cohort of patients that were being treated as part of a registrar training post, some were involved in a functional / fixed treatment plan. This means that they had some previous experience with removable orthodontic appliances and in general they had good arch alignment which is a recommendation for this treatment modality.

It was decided to recruit adolescent patients to the study as the majority of patients receiving orthodontic treatment fall into this age category and they were unlikely to have experienced significant periodontal disease which may alter the soft tissue architecture and affect plaque accumulation. Many of the studies which have found positive correlations between crowding and plaque, however, have involved older patients and quite often young adults over 20 years old [Chung *et al*, 2000; Ngom *et al*, 2006; Ainamo, 1972; Griffiths *et al*, 1981]. This cohort of patients may be more susceptible to gingivitis and a positive correlation between crowding and specific fluorescent bacteria may be more apparent and significant in an older age group.

8.2.2 Timing of samples

The data collected at each appointment were not at standardised appointment times. This was to avoid increasing the inconvenience to the patients which might have prevented them from participating in the study. It may be that plaque levels measured varied according to the time of day in relation to a patient's oral hygiene practices. This should have had little effect in our study, however, as the Toothcare^{TM} light highlights fluorescent plaque which is usually indicative of mature plaque which has been present for at least 3 days [Coulthwaite *et al*, 2005; van der Veen *et al*, 2006].

8.2.3 Follow-up period

Ideally it would be preferable to follow-up the participants after the appliances have been debonded and during the retention period to see if there is an alteration to plaque levels again once the confounding factor of the orthodontic appliances has been removed. It would still be difficult to infer, however, if any changes were related to improved dental alignment or due to behavioural changes implemented over 2 years of treatment.

8.2.4 Test re-test of Toothcare^{TM} device

The first reliability test of the Toothcare^{TM} device only involved a short time delay which may allow recall of the previous score to bias the results. The subsequent use of the QLF-D^{TM} camera, however, allowed a more robust measure of reliability.

8.3 Subjectivity of methods

8.3.1 Repeatability Tests

The criteria for repeatability tests were met for all measures:

- Same measurement procedure used
- Same observer involved
- Same instrument used under the same conditions
- Same location
- Repetition carried out over a short time

8.3.2 Use of Toothcare™ device

All plaque charts were completed at the chair side by the same observer. The plaque index used estimates the area of plaque coverage and this is open to some subjectivity. By maintaining the same observer, however, consistency in plaque scoring was achieved as indicated by the high κ score.

The Toothcare™ device was very easy to use and did not require a large amount of training. It has also been shown in a previous study [Thomas, 2010] that it detects a greater amount of plaque than QLF or white light. Although previous research with QLF suggests that distinct fluorescence can be seen which may be red, orange or green, only orange plaque was detected in this study. This may be due to the complex nature of a plaque biofilm the presence of simultaneous fluorescent colours within one sample rather than distinct bands of colour. The ability of Toothcare™ to detect fluorescent plaque, however, is extremely useful when investigating plaque associated oral disease as it is more likely to delineate pathogenic bacteria and, therefore, it may be a useful method for screening patients at risk of developing such diseases.

The QLF-D Biluminator^{TM} is based on similar technology and produces a recordable image which can be stored and analysed multiple times. The 2-dimensional image, however, may affect the accuracy in determining quantities on a 3-dimensional surface. It also requires more training to use effectively and diagnosis may be improved with greater contrast in the QLF image obtained.

8.3.3 Confounding factors

The inability of this study to detect a positive correlation between plaque and crowding may be due to a number of potentially confounding factors that were not controlled for in this longitudinal observational study. These include:

- Inherent behavioural factors within the patient
- The presence of an orthodontic appliance and elastomeric modules
- The timing of each observation
- The routine regular reinforcement of oral hygiene advice during an extensive orthodontic treatment period

8.3.4 Statistical analysis

Due to the small sample size and, therefore, skewed distribution of the data non-parametric statistical tests were chosen. These tests are useful as they make fewer assumptions about the data, however, they are not as powerful as the parametric equivalents. For this reason they may be more likely to find false negative results and overlook a significant relationship which may be present.

9. CONCLUSION

This study aimed to determine if there was a correlation between crowding and plaque accumulation in an orthodontic population and also tested a novel device for the detection of plaque *in vivo*.

The conclusions for this study are summarised below:

1. Plaque levels in the orthodontic population studied were not positively correlated with the degree of irregularity of the anterior teeth. In some cases the association was an inverse association, however, the magnitude of this relationship appears to be very small.
2. Although a correlation was not found, there was a general pattern of deterioration in oral hygiene following the placement of a fixed appliance. This is to be expected, however, it must be emphasised to patients that oral hygiene will become more difficult and thorough demonstration of tooth brushing and interdental cleaning as well as cleaning three times a day should be recommended.
3. The Toothcare™ light is an extremely useful chair side tool for detecting the presence of plaque and combined with a simple scoring system shows good reliability. The development of the QLF-D camera based on the same principles but producing a record of the fluorescent image will further increase the reproducibility of this method for epidemiological studies involving plaque.
4. In this study hand scoring of Little's Index was more accurate than digitising the points on a reflex metrograph.

10. Future Recommendations

1. The development of the QLF-D Biluminator™ could be used in conjunction with planimetric computer methods to measure surface area of plaque accumulation which would provide quantitative data as opposed to the ordinal data derived from most current plaque indices.

2. An alternative to measuring the surface area of the teeth affected may be to collect and measure the dry weight of plaque to determine if the 'depth' of deposit is altered in a crowded dentition.

3. A study of changes in plaque accumulation of those patients who routinely receive dental health education prior to orthodontic treatment and those who do not may indicate a difference in pattern / behaviour.

4. The Toothcare™ device could be considered as an oral hygiene aid for patients as it is relatively simple to use although a comparison to methods using disclosing dyes may be adivisable.

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12. APPENDICES

Appendix A

UNIVERSITY OF LIVERPOOL

INFORMATION SHEET

Investigation of the relationship between tooth irregularity and plaque in an orthodontic population

Your child is being invited to take part in a research study which is looking at whether overlapping / irregular teeth can lead to differences in the level of cleanliness of the teeth. Before you decide for your child to take part in the study please take time to read this information sheet. Please ask us if there is anything that is not clear or if you would like further information.

Toothcare™ is a hand held light which allows accurate detection of plaque (food debris) on teeth. The blue light enables plaque debris to be seen as fluorescent areas on the teeth. It is also able to show enamel damage, which causes permanent marks on teeth, at an earlier stage than by eye sight alone. This device will help us to monitor the health of a patient's teeth in a more accurate way than possible before and allow us to manage any risks of permanent damage to the teeth.

The investigation itself will not involve any alteration to the orthodontic treatment apart from slight extension of appointment times.

What is the purpose of the study?

It has often been suggested that straightening teeth through orthodontic treatment should make it easier to clean them. It hasn't, however, been possible to show this substantially in previous studies. This study hopes to find out more information about crowded teeth and tooth cleaning during brace treatment so that accurate advice can be given to patients in the future.

Has the study been approved?

Approved by Liverpool Research Ethics Committee 1 in October 2009

Who will be conducting the study?

This study is being run by Prof. Susan Higham (Professor of Oral Biology), Prof. Neil Pender (Professor of Orthodontics) and Anika Maini (Specialist Registrar in Orthodontics).

Why has my child been chosen to take part?

We are looking for healthy volunteers who will be starting fixed brace treatment.

What will happen if my child takes part?

Before placing the fixed brace the amount and colour of plaque on the front 12 teeth (upper and lower) will be measured using the Toothcare™ light and marked on a chart. All plaque will be cleaned off the teeth to check for any damage and this will also be recorded. Additionally a mould or impression of both upper and lower teeth will be taken to measure crowding. These measures will be repeated at each appointment for a period of a year. This should lengthen appointment time by no more than 15 minutes.

How long will the study last?

Your child will be monitored for at most, 12 months of fixed brace treatment.

What if I don't want my child to take part?

Your child's treatment will continue as normal. You should not feel obliged to consent to this study, and you do not have to give a reason if you don't want to. Also, if you decide to consent to your child taking part but later decide not to, you can withdraw consent at any time without needing to give a reason.

What if I have a question or there is a problem on the trial?

Any questions or problems can be discussed with any of the dentists running the study in the hospital. The principal investigator is Anika Maini, Orthodontic SpR, Liverpool University Dental Hospital, Pembroke Place, L3 5PS. Tel: 0151 706 5238

How will my child's data be collected and managed?

All information collected about your child will be processed and analysed by the research staff involved in the study. Data will be stored for ten years. As soon as necessary data has been collected any identifying information will be removed and replaced by a code number. The person responsible for security access to the data is Professor Neil Pender, the Chief Investigator of the study.

What do I do if I'm happy for my child to take part?

If you would like your child to take part, please sign all the relevant sections of the consent form that you will have been provided with.

THANK YOU FOR TAKING THE TIME TO READ THIS

Patient Identification Number for trial:

CONSENT FORM

Research Project: The relationship between tooth irregularity and plaque.

Researcher(s): Anika Maini

Please initial box

1. I confirm that I have read and understand the information sheet dated October 2009 (version 3.1) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

2. I understand that my child's participation is voluntary and that I am free to withdraw them at any time without giving any reason, without their medical care or legal rights being affected.

3. I understand that the data collected during the study will be analysed by the study investigators. I give permission for these individuals to have access to my child's records.

4. I agree to my child taking part in the above study.

Parent / Guardian	Date	Signature

Name of clinician taking consent	Date	Signature

The contact details of lead Researcher (Chief Investigator) are:
Professor Neil Pender
Professor of Orthodontics
LUDH Pembroke Place
Liverpool
L3 5PS
Tel: 01517065210
n.pender@liverpool.ac.uk

INFORMATION SHEET FOR UNDER 16

Investigation of the relationship between tooth irregularity and plaque in an orthodontic population

You are being asked to be part of a project to see if crooked teeth can affect toothbrushing. Before you decide to join the project please read this information sheet. Please ask us if you have any questions.

The project will not change your brace treatment. It will only make your visits a few minutes longer.

What is the point of the project?

We want to find out if having crooked teeth makes it more difficult to keep them clean.

Why do I need to take part?

You are the right age and are about to have your braces fitted.

What will happen if I say yes?

Before fitting your brace we will use a special light to find any areas where food has not been cleaned away. **Toothcare**™ is a blue light which shows up any food still stuck on the teeth. It also helps to show us if there are any holes appearing in your teeth.

We will use the light at every visit after your brace is fitted. This way we can see if as your teeth straighten they become easier to clean. We will also need to take small moulds or impressions of your front teeth.

How long is the project?

The project will last for 12 months.

What if I don't want to take part?

You will still start your brace treatment. You do not have to take part. You don't have to tell us why if you don't want to.

What if I have a question or there is a problem?

You can ask me any questions you want and I will try my best to answer them. Also, if you decide later that you don't want to take part anymore you can stop without having to say why.

UNIVERSITY OF LIVERPOOL

ASSENT FORM FOR UNDER 16s

Research Project: The relationship between tooth irregularity and plaque.

Please circle YES or NO

1.	I have read the information sheet (October 2009 version 2.1)	YES	NO
2.	The project has been explained to me.	YES	NO
3.	I have been able to ask questions and have had these answered.	YES	NO
4.	I understand what the project is about and what I need to do.	YES	NO
5.	I understand I can stop taking part at any time.	YES	NO
6.	I am happy to take part in this project.	YES	NO

If you are happy to take part please write your name below

Name Date

Name of parent or guardian

Name Date Signature

Name of researcher

Name Date Signature

The contact details of lead Researcher (Chief Investigator) are:
Professor Neil Pender, Professor of Orthodontics
LUDH Pembroke Place
Liverpool, L3 5PS
Tel: 01517065210
n.pender@liverpool.ac.uk

INFORMATION SHEET

Investigation of the relationship between tooth irregularity and plaque in an orthodontic population

You are being invited to take part in a research study which is looking at whether overlapping / irregular teeth can lead to differences in the level of cleanliness of the teeth. Before you decide to take part in the study please take time to read this information sheet. Please ask us if there is anything that is not clear or if you would like further information.

Toothcare™ is a hand held device which allows accurate detection of plaque (food debris) on teeth. It emits a blue light which, when viewed through a filter, enables plaque debris to be seen as fluorescent areas on the teeth. It is also able to show enamel damage, which causes permanent marks on teeth, at an earlier stage than by eye sight alone. This device will help us to monitor the health of a patient's teeth in a more accurate way than possible before and allow us to manage any risks of permanent damage to the teeth.

The investigation itself will not involve any alteration to the orthodontic treatment apart from slight extension of appointment times.

What is the purpose of the study?

It has often been suggested that straightening teeth through orthodontic treatment should make it easier to clean them. It hasn't, however, been possible to show this substantially in previous studies. This study hopes to find out more information about crowded teeth and tooth cleaning during brace treatment so that accurate advice can be given to patients in the future.

Has the study been approved?

(awaiting approval from Liverpool local research ethics committee)

Who will be conducting the study?

This study is being run by Prof. Susan Higham (Professor of Oral Biology), Prof. Neil Pender (Professor of Orthodontics) and Anika Maini (Specialist Registrar in Orthodontics).

Why have I been chosen to take part?

We are looking for healthy volunteers who will be starting fixed brace treatment.

What will happen if I take part?

Before placing the fixed brace the amount and colour of plaque on the front 12 teeth (upper and lower) will be measured using the Toothcare™ light and marked on a chart. All plaque will be cleaned off the teeth to check for any damage and this will also be recorded. Additionally a mould or impression of both upper and lower teeth will be taken to measure crowding. These measures will be repeated at each appointment for a period of a year. This should lengthen appointment time by no more than 15 minutes.

How long will the study last?

You will be monitored for 12 months of fixed brace treatment.

What if I don't want to take part?

Your treatment will continue as normal. You should not feel obliged to take part, and you do not have to give a reason if you don't want to. Also, if you decide to take part but later decide not to you can withdraw from the study at any time without needing to give a reason.

What if I have a question or there is a problem on the trial?

Any questions or problems can be discussed with any of the dentists running the study in the hospital. The principal investigator is Anika Maini, Orthodontic SpR, Liverpool University Dental Hospital, Pembroke Place, L3 5PS. Tel: 0151 706 5238

How will my data be collected and managed?

All information collected about you will be processed and analysed by the research staff involved in the study. Data will be stored for ten years. As soon as necessary data has been collected any information identifying you will be removed and replaced by a code number. The person responsible for security access to your data is Professor Neil Pender, the Chief Investigator of the study.

What do I do if I want to take part?

If you would like to take part, please sign all the relevant sections of the consent form that you will have been provided with.

THANK YOU FOR TAKING THE TIME TO READ THIS

UNIVERSITY OF
LIVERPOOL

Patient Identification Number for trial:

CONSENT FORM

Research Project: The relationship between tooth irregularity and plaque.

Researcher(s): Anika Maini

Please initial box

1. I confirm that I have read and understand the information sheet dated August 2009 (version 1.1) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand that the data collected during the study will be analysed by the study investigators. I give permission for these individuals to have access to my records. ☐

4. I agree to take part in the above study. ☐

Patient	Date	Signature
Name of clinician taking consent	Date	Signature

The contact details of lead Researcher (Chief Investigator) are:
Professor Neil Pender
Professor of Orthodontics
LUDH Pembroke Place
Liverpool
L3 5PS
Tel: 01517065210
n.pender@liverpool.ac.uk

INFORMATION SHEET

Investigation of the relationship between tooth irregularity and plaque in an orthodontic population

Your child is being invited to take part in a research study to compare 2 methods of detecting plaque (food debris) on teeth. Before you decide for your child to take part in the study please take time to read this information sheet. Please ask us if there is anything that is not clear or if you would like further information.

Toothcare^{TM} is a hand held light which allows accurate detection of plaque (food debris) on teeth. The blue light enables plaque debris to be seen as fluorescent areas on the teeth. It is also able to show enamel damage, which causes permanent marks on teeth, at an earlier stage than by eye sight alone. This device will help us to monitor the health of a patient's teeth in a more accurate way than possible before and allow us to manage any risks of permanent damage to the teeth.

Quantitative Light Fluorescence – digital (**QLF-D^{TM})** is a digital camera which records a picture similar to that seen with the **Toothcare^{TM}** light.

What is the purpose of the study?

The study is to compare using the **Toothcare^{TM}** light to the **QLF-D^{TM}** photograph and determine if we can accurately measure plaque with the hand-held light.

Has the study been approved?

Approved by Liverpool Research Ethics Committee 1 in October 2009

Who will be conducting the study?

This study is being run by Prof. Susan Higham (Professor of Oral Biology), Prof. Neil Pender (Professor of Orthodontics) and Anika Maini (Specialist Registrar in Orthodontics).

Why has my child been chosen to take part?

We are looking for healthy volunteers before they start fixed brace treatment.

What will happen if my child takes part?

At the start of the appointment the amount and colour of plaque on the front 12 teeth (upper and lower) will be measured using the Toothcare^{TM} light and marked on a chart. Then a photograph will be taken of the front 12 teeth.

How long will the study last?

The data will be collected for one appointment only.

What if I don't want my child to take part?

Your child's treatment will continue as normal. You should not feel obliged to consent to this study, and you do not have to give a reason if you don't want to. Also, if you decide to consent to your child taking part but later decide not to, you can withdraw consent at any time without needing to give a reason.

What if I have a question or there is a problem on the trial?

Any questions or problems can be discussed with any of the dentists running the study in the hospital. The principal investigator is Anika Maini, Orthodontic SpR, Liverpool University Dental Hospital, Pembroke Place, L3 5PS. Tel: 0151 706 5238

How will my child's data be collected and managed?

All information collected about your child will be processed and analysed by the research staff involved in the study. Data will be stored for ten years. As soon as necessary data has been collected any identifying information will be removed and replaced by a code number. The person responsible for security access to the data is Professor Neil Pender, the Chief Investigator of the study.

What do I do if I'm happy for my child to take part?

If you would like your child to take part, please sign all the relevant sections of the consent form that you will have been provided with.

THANK YOU FOR TAKING THE TIME TO READ THIS

Patient Identification Number for trial:

CONSENT FORM

Research Project: The relationship between tooth irregularity and plaque.

Researcher(s): Anika Maini

Please initial box

1. I confirm that I have read and understand the information sheet dated June 2011 (version 4.1) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

2. I understand that my child's participation is voluntary and that I am free to withdraw them at any time without giving any reason, without their medical care or legal rights being affected.

3. I understand that the data collected during the study will be analysed by the study investigators. I give permission for these individuals to have access to my child's records.

4. I agree to my child taking part in the above study.

Parent / Guardian	Date	Signature
Name of clinician taking consent	Date	Signature

The contact details of lead Researcher (Chief Investigator) are:
Professor Neil Pender
Professor of Orthodontics
LUDH Pembroke Place
Liverpool
L3 5PS
Tel: 01517065210
n.pender@liverpool.ac.uk

INFORMATION SHEET FOR UNDER 16

Investigation of the relationship between tooth irregularity and plaque in an orthodontic population

You are being asked to be part of a project to look at a new way which shows if food is stuck on your teeth. It only requires you to take part once and will not affect any of your future treatment.

What is the point of the project?

We want to compare a photo and a new method to show any food stuck on your teeth.

Why do I need to take part?

You are the right age and have not had your teeth straightened as yet.

What will happen if I say yes?

We will use a special light to find any areas where food has not been cleaned away. **Toothcare™** is a blue light which shows up any food still stuck on the teeth. The Quantitative Light Fluorescence-Digital (**QLF-D**) camera also shows up any food still stuck on the teeth and we will use this camera to take a photograph of your teeth and compare both methods.

We will only do this once and it will not affect any of your future visits.

What if I don't want to take part?

You do not have to take part. You don't have to tell us why if you don't want to.

What if I have a question or there is a problem?

You can ask me any questions you want and I will try my best to answer them. Also, if you decide later that you don't want to take part anymore you can stop without having to say why.

UNIVERSITY OF LIVERPOOL

ASSENT FORM FOR UNDER 16s

Research Project: The relationship between tooth irregularity and plaque.

Please circle YES or NO

1. I have read the information sheet (June 2011 version 5.1)	YES	NO
2. The project has been explained to me.	YES	NO
3. I have been able to ask questions and have had these answered.	YES	NO
4. I understand what the project is about and what I need to do.	YES	NO
5. I understand I can stop taking part at any time.	YES	NO
6. I am happy to take part in this project.	YES	NO

If you are happy to take part please write your name below

____________________ ________________

Name Date

Name of researcher

____________________ ________________ ____________

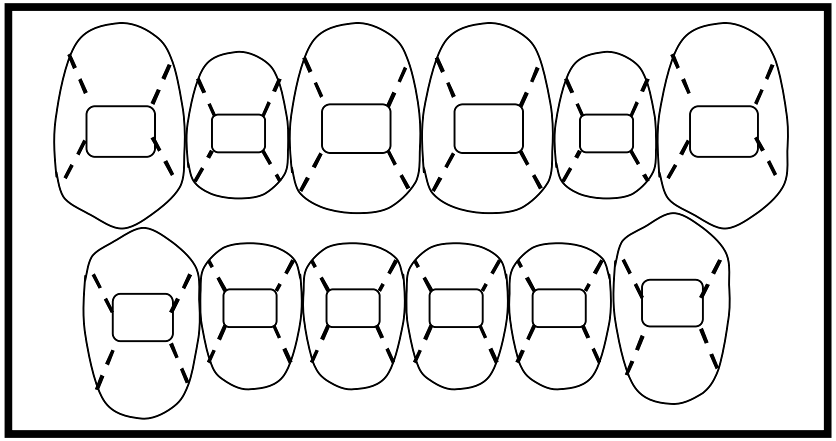
Name Date Signature

The contact details of lead Researcher (Chief Investigator) are:
Professor Neil Pender, Professor of Orthodontics
LUDH Pembroke Place
Liverpool, L3 5PS
Tel: 01517065210
n.pender@liverpool.ac.uk

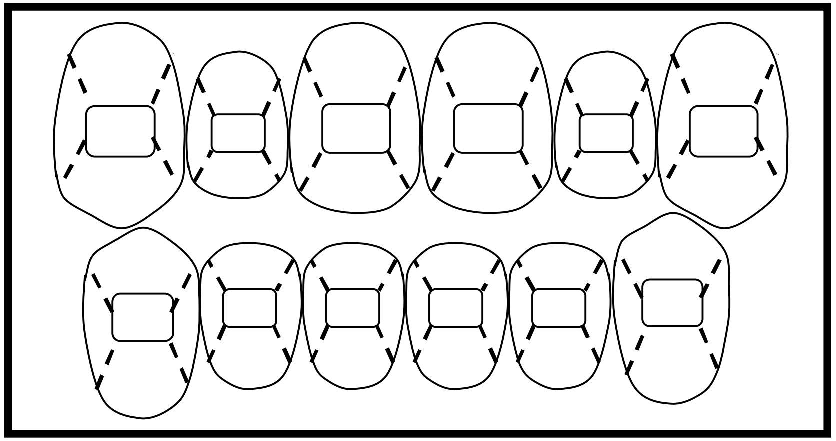
Appendix B

PLAQUE

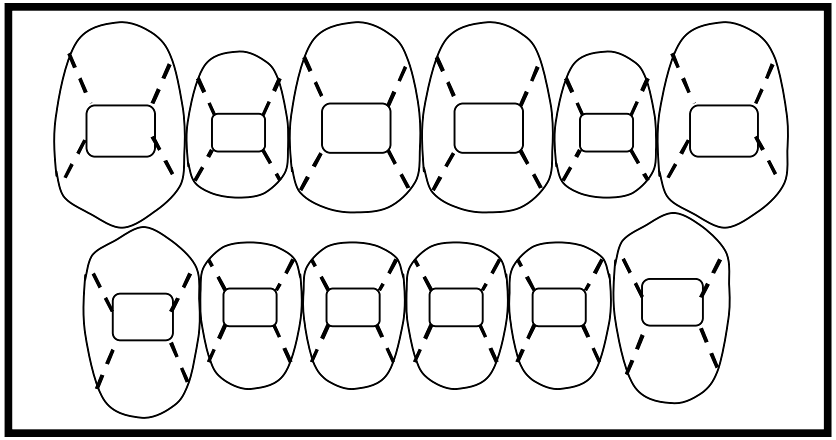
1. Pre-bond-up Date:



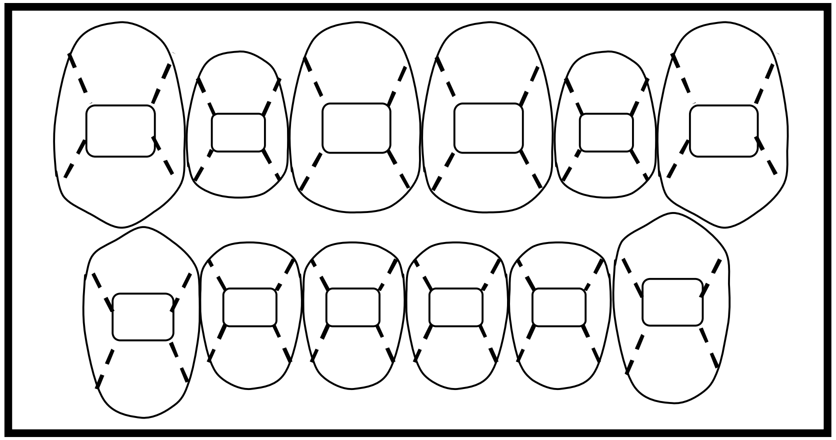
2. 1st visit Date:



3. 2nd visit Date:



4. 4th visit Date:



ber:.. **Appendix C**

Little's Index Record Sheet

Pre-Bond-Up

3-2	2-1	1-1	1-2	2-3
3-2	2-1	1-1	1-2	2-3

T =................

Visit 1

3-2	2-1	1-1	1-2	2-3
3-2	2-1	1-1	1-2	2-3

T =................

Visit 2

3-2	2-1	1-1	1-2	2-3
3-2	2-1	1-1	1-2	2-3

T =................

Visit 3

3-2	2-1	1-1	1-2	2-3
3-2	2-1	1-1	1-2	2-3

T =................

Visit 4

3-2	2-1	1-1	1-2	2-3
3-2	2-1	1-1	1-2	2-3

T =................

Visit 5

3-2	2-1	1-1	1-2	2-3
3-2	2-1	1-1	1-2	2-3

T =................

Visit 6

3-2	2-1	1-1	1-2	2-3
3-2	2-1	1-1	1-2	2-3

T =................

Visit 7

3-2	2-1	1-1	1-2	2-3
3-2	2-1	1-1	1-2	2-3

T =................

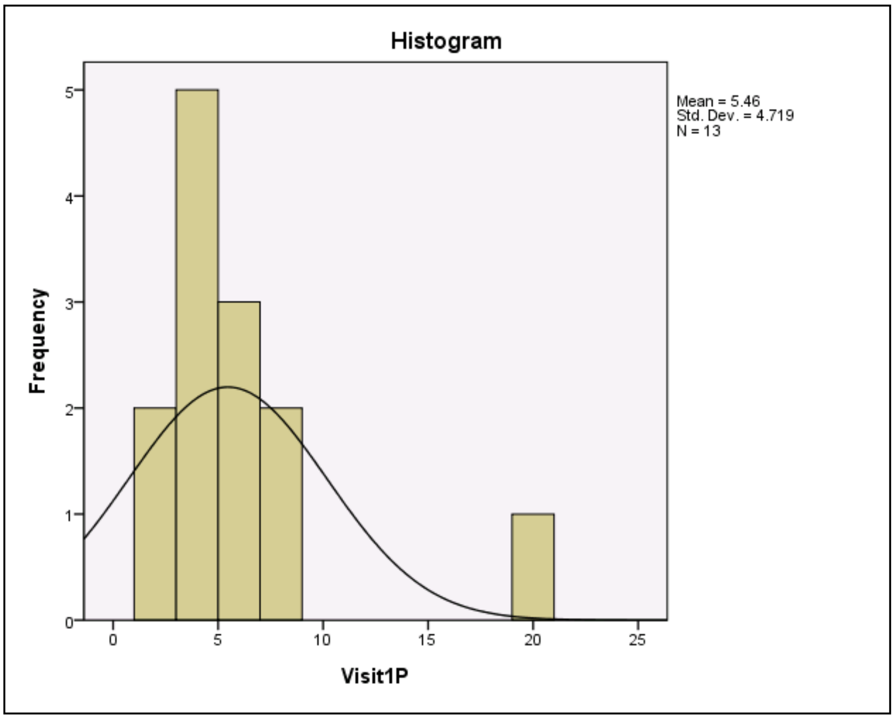


Figure 12.1: Visit 1 Plaque distribution

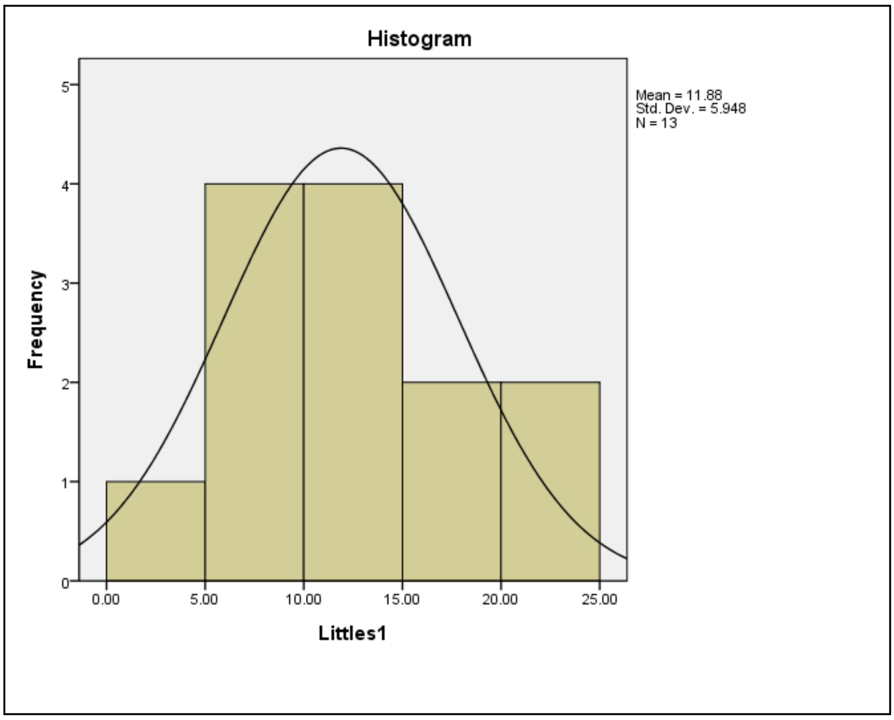


Figure 12.2: Visit 1 Little's Index distribution

Appendix E

REPEATABILITY DATA OF FIRST AND SECOND LITTLE'S SCORES BY HAND

Reading	Littles 1	Littles 2	Difference	Reading	Littles 1	Littles 2	Difference
1	0	0	0	41	0	0.6	0.6
2	1	0.9	-0.1	42	0	0	0
3	0.3	0.3	0	43	0	0.5	0.5
4	0.45	0.44	-0.01	44	0.5	0	0.5
5	10	9.6	-0.4	45	0	0	0
6	0	0	0	46	0	0	0
7	3.2	3.25	0.05	47	1	0.6	-0.4
8	0	0	0	48	6.2	6.6	0.4
9	0.4	0.6	0.2	49	1.5	1.8	0.3
10	0.21	0.21	0	50	1.5	1.9	0.4
11	0	0	0	51	1.1	1	-0.1
12	0	0	0	52	1	0.9	-0.1
13	0.16	0.15	-0.01	53	1.4	0.9	-0.5
14	0	0	0	54	0	0.5	0.5
15	2.2	2.24	0.04	55	1.5	1.1	-0.4
16	0	0	0	56	0.8	0.55	-0.25
17	1	1.2	0.2	57	0	0	0
18	0.7	1.05	0.35	58	0.4	0.4	0
19	0	0	0	59	0	0.3	0.3
20	0.6	0.85	0.25	60	4.1	4	-0.1
21	0	0	0	61	4.6	4.5	-0.1
22	0.73	0.72	-0.01	62	0	0	0
23	0	0	0	63	4.2	3.5	-0.7
24	0.3	0.3	0	64	4.5	4.5	0
25	0.4	0.5	0.1	65	0	0.4	0.4
26	0	0	0	66	1.12	1.01	-0.11
27	0	0	0	67	0	0	0
28	0	0	0	68	0	0	0
29	0.3	0	-0.3	69	0	0	0
30	0	0	0	70	4.8	5.1	0.3
31	0.55	0.55	0	71	1.1	1.5	0.4
32	0	0	0	72	0	0	0
33	0.1	0	-0.1	73	0	0	0
34	0	0	0	74	4.1	4	-0.1
35	0.6	0	-0.6	75	0	0	0
36	0	0	0	76	0	0	0
37	0	0	0	77	0.5	0.9	0.4
38	0	0.1	0.1	78	1.25	1.4	0.15
39	0	0	0	79	0	0	0
40	1.3	1.5	0.2				

Appendix F

REPEATABILITY DATA OF FIRST AND SECOND LITTLE'S SCORE USING REFLEX METROGRAPH

Reading	Reflex 1	Reflex 2	Difference	Reading	Reflex 1	Reflex 2	Difference
1	1.45	0.79	-0.66	**41**	5.04	5.13	0.09
2	1.68	2.01	0.33	**42**	0.0	0.0	0
3	1.78	1.42	-0.36	**43**	1.65	2.11	0.46
4	5.12	4.89	-0.23	**44**	1.41	2.07	0.66
5	8.71	8.79	0.08	**45**	0.50	0.38	-0.12
6	2.46	2.83	0.37	**46**	1.07	1.16	0.09
7	1.30	0.89	-0.41	**47**	0.40	0.52	0.12
8	2.50	2.07	-0.43	**48**	0.0	0.0	0
9	0.0	0.0	0	**49**	0.78	0.97	0.19
10	1.39	1.42	0.03	**50**	0.87	1.17	0.3
11	5.73	6.05	0.32	**51**	3.21	3.99	0.78
12	1.40	0.96	-0.44	**52**	4.66	4.95	0.29
13	0.33	0.52	0.19	**53**	1.35	1.04	-0.31
14	0.0	0.0	0	**54**	3.70	3.99	0.29
15	4.50	4.29	-0.21	**55**	8.64	8.49	-0.15
16	0.0	0.0	0	**56**	0.79	0.86	0.07
17	0.0	0.0	0	**57**	0.0	0.0	0
18	0.85	0.99	0.14	**58**	0.0	0.0	0
19	1.30	1.20	-0.1	**59**	3.10	3.14	0.04
20	0.0	0.0	0	**60**	1.80	2.57	0.77
21	4.77	4.99	0.22	**61**	1.80	1.79	-0.01
22	2.36	3.59	1.23	**62**	0.0	0.0	0
23	0.56	0.0	-0.56	**63**	0.0	0.0	0
24	1.54	2.10	0.56	**64**	0.0	0.0	0
25	3.08	3.04	-0.04	**65**	0.70	0.98	0.28
26	0.0	0.0	0	**66**	0.20	0.0	-0.2
27	1.73	1.59	-0.14	**67**	0.84	1.51	0.67
28	1.02	1.24	0.22	**68**	3.43	3.09	-0.34
29	1.93	1.56	-0.37	**69**	5.30	5.54	0.24
30	3.43	3.63	0.2	**70**	1.85	1.64	-0.21
31	3.92	4.24	0.32	**71**	1.01	1.01	0
32	2.48	2.54	0.06	**72**	0.80	0.67	-0.13
33	0.0	0.0	0	**73**	1.79	1.99	0.2
34	2.15	2.31	0.16	**74**	0.54	0.61	0.07
35	3.55	3.07	-0.48	**75**	1.65	1.79	0.14
36	1.91	1.56	-0.35	**76**	1.04	1.68	0.64
37	0.73	0.62	-0.11	**77**	0.0	0.0	0
38	0.0	0.0	0	**78**	0.77	0.66	-0.11
39	0.89	0.99	0.1	**79**	0.38	0.0	-0.38
40	1.09	1.80	0.71				

Appendix G

COMPARISON OF LITTLE'S INDEX SCORES TAKEN BY HAND AND USING THE REFLEX METROGRAPH

Study No	Visit No	Hand (mm)	Reflex (mm)	Study No	Visit No	Hand (mm)	Reflex (mm)
1	1	10.90	14.37	**1**	3	11.55	14.61
2	1	6.20	7.65	**2**	3	2.00	6.53
3	1	7.55	9.83	**3**	3	1.95	2.83
4	1	21.60	25.45	**4**	3	11.10	11.31
5	1	10.15	11.72	**5**	3	6.65	7.48
6	1	17.00	18.11	**6**	3	18.70	18.33
7	1	6.30	13.26	**7**	3	2.80	8.84
8	1	3.90	9.02	**8**	3	.70	1.66
9	1	17.20	20.42	**9**	3	11.20	11.46
10	1	12.90	14.11	**10**	3	.0	.96
11	1	6.60	10.32	**11**	3	.0	.0
12	1	21.70	26.39	**12**	3	17.60	17.56
13	1	12.50	13.39	**13**	3	.0	.56
1	2	14.50	16.32	**1**	4	11.60	10.79
2	2	2.45	4.31	**2**	4	2.40	5.17
3	2	2.50	4.97	**4**	4	11.00	11.01
4	2	13.95	16.66	**5**	4	2.50	4.20
5	2	8.25	9.65	**6**	4	.60	.63
6	2	16.90	17.43	**7**	4	1.00	8.86
7	2	6.05	13.14	**8**	4	.0	.0
8	2	1.35	2.50	**9**	4	5.30	6.32
9	2	15.30	16.72	**10**	4	.35	1.36
10	2	.0	2.16	**12**	4	12.90	12.68
11	2	.0	7.86	**13**	4	.0	.0
12	2	20.40	21.49	**9**	5	.60	.94
13	2	2.25	1.60	**12**	5	12.40	12.13

Appendix H

TABLE OF OBSERVED FREQUENCIES OF PLAQUE SCORE AGREEMENT USING TOOTHCARE^{TM} (KAPPA TABLE)

Observed frequencies	0	1	2	3	Total
0	287	5	0	0	292
1	1	47	4	0	52
2	0	0	24	0	24
3	0	0	0	0	0
Total	288	52	28	0	***368***

$$\frac{\left[\frac{287 + 47 + 24 + 0}{348}\right] - \left[\frac{228.5 + 7.3 + 1.8 + 0}{348}\right]}{1 - \left[\frac{228.5 + 7.3 + 1.8 + 0}{348}\right]}$$

Kappa = 0.92

Appendix I

TABLE OF OBSERVED FREQUENCIES OF PLAQUE SCORE SENSITIVITY BETWEEN QLF-D BILUMINATOR^{TM} AND TOOTHCARE^{TM} (KAPPA TABLE)

Observed frequencies	0	1	2	3	Total
0	288	2	0	0	290
1	17	35	1	0	53
2	3	7	17	0	27
3	0	0	0	2	2
Total	308	44	18	2	***372***

$$\frac{\left[\frac{288 + 35 + 17 + 2}{372}\right] - \left[\frac{240.1 + 6.3 + 1.3 + 0.01}{372}\right]}{1 - \left[\frac{240.1 + 6.3 + 1.3 + 0.01}{372}\right]}$$

Kappa = 0.76

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The median of differences between Littles1 and Littles2 equals 0.	Related-Samples Wilcoxon Signed Rank Test	.007	Reject the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

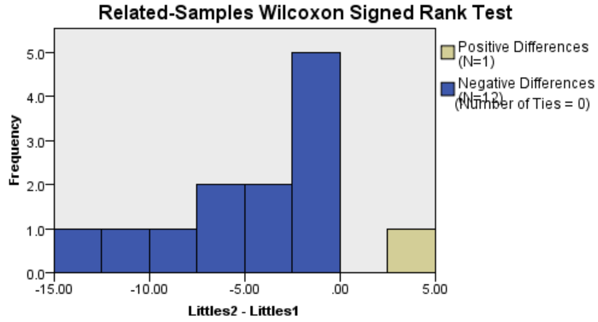


Figure 12.3: Difference in crowding from T1- T2

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The median of differences between Littles2 and Littles3 equals 0.	Related-Samples Wilcoxon Signed Rank Test	.013	Reject the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

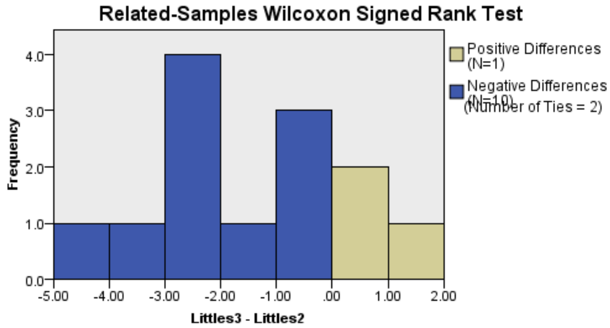


Figure 12.4: Difference in crowding from T2 to T3

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The median of differences between Littles3 and Littles4 equals 0.	Related-Samples Wilcoxon Signed Rank Test	.646	Retain the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

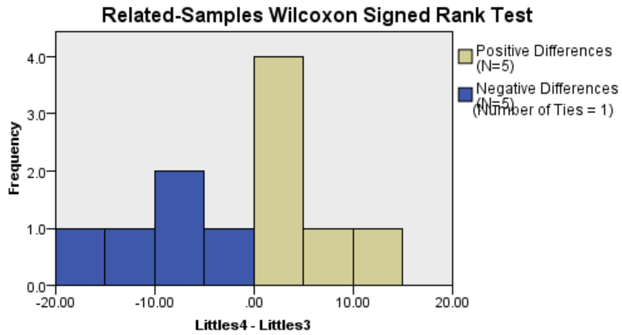


Figure 12.5: Difference in crowding T3-T4

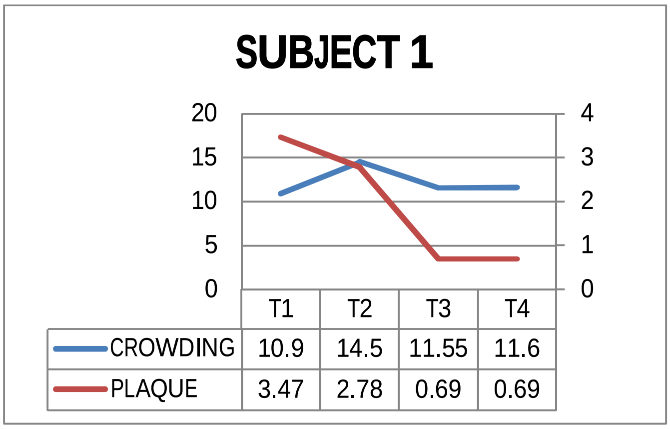


Figure 12.6: Patient 1 trends of plaque accumulation and anterior crowding over time.

The crowding for this patient worsened slightly between the first and second visit before starting to reduce whilst the plaque accumulation showed a consistently reducing trend. Despite the degree initial of irregularity, the plaque accumulation was very low and oral hygiene was good throughout treatment.

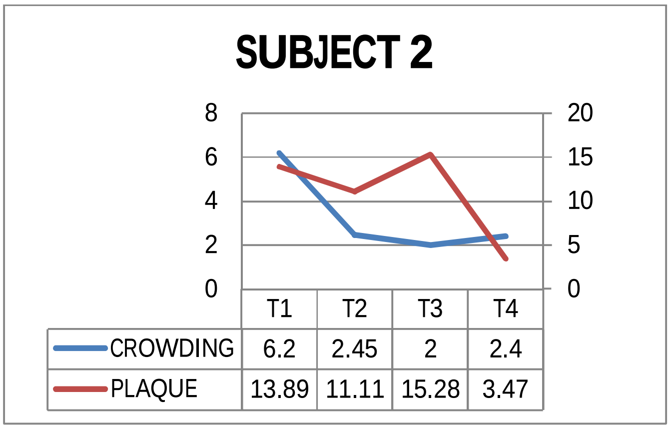


Figure 12.7: Patient 2 trends of plaque accumulation and anterior crowding over time.

There was a general downward trend in terms of crowding for this subject and, again, the plaque accumulation did not mirror the change in crowding as it worsened between the second and third visits before eventually reducing by the fourth visit.

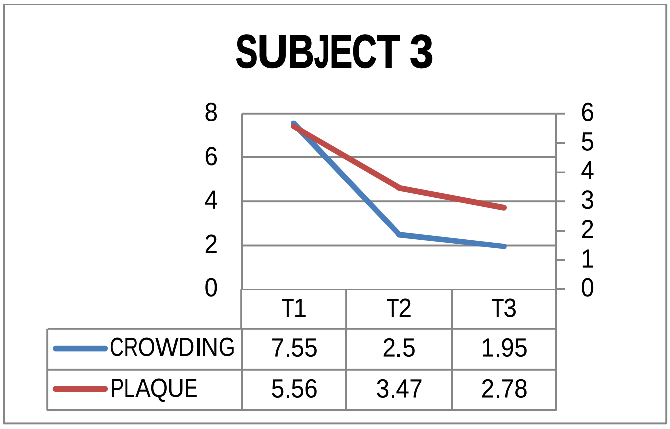


Figure 12.8: Patient 3 trends of plaque accumulation and anterior crowding over time.

Both plaque and crowding appeared to have a similar trend, both reducing with time. The majority of change for both variables was between the first and second compared to the second and third visits.

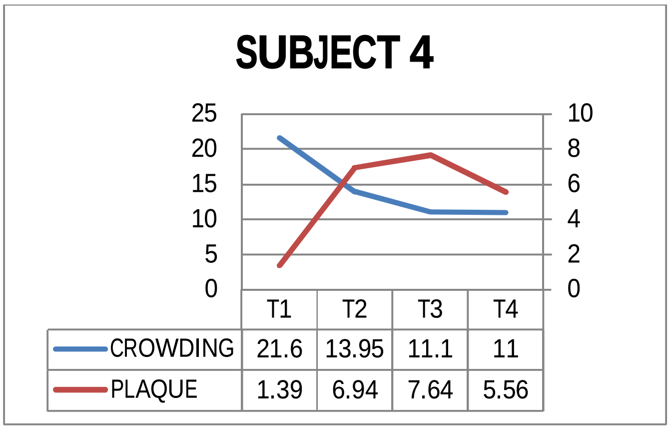


Figure 12.9: Patient 4 trends of plaque accumulation and anterior crowding over time

The pattern for the variables in this patient appear to be mirror images as instead of the plaque levels improving as crowding reduced, the opposite happens and the plaque level increase after the appliance is bonded and even with the improvement in irregularity, the plaque level does not return to the pre-treatment low level.

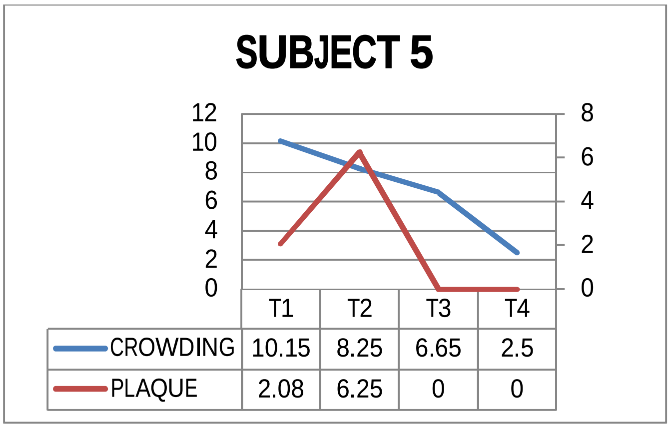


Figure 12.10: Patient 5 trends of plaque accumulation and anterior crowding over time

Whilst there is a consistent downward trend for the irregularity of the teeth, the trend for plaque accumulation is much more variable. Again, there is a worsening in the plaque score in the visit after the appliance is fitted, however, this does improve markedly over the following two visits.

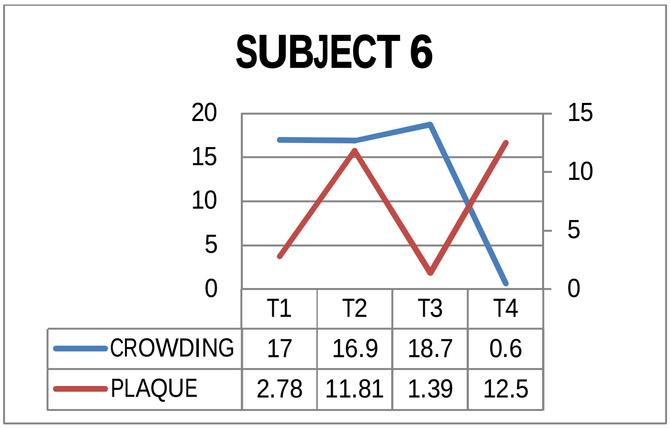


Figure 12.11: Patient 6 trends of plaque accumulation and anterior crowding over time.

The trend of plaque accumulation is very variable in this patient with significant changes between appointments, initially worsening significantly at the second appointment before improving only to increase again at the last visit, despite the irregularity score reducing to almost 0mm.

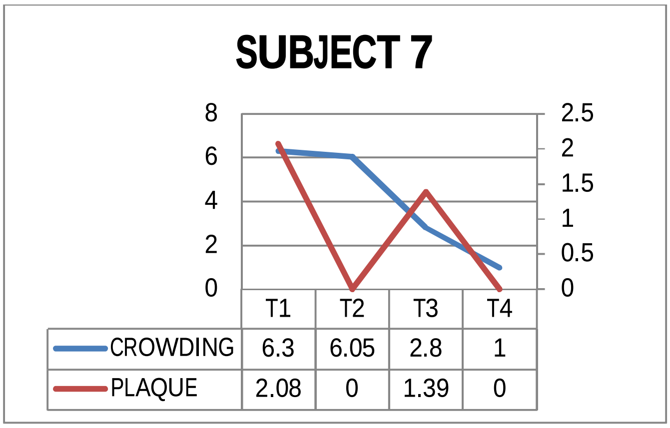


Figure 12.12: Patient 7 trends of plaque accumulation and anterior crowding over time.

There is a general downward trend with reference to the irregularity score over time, the plaque score, however, is generally low throughout the observation period.

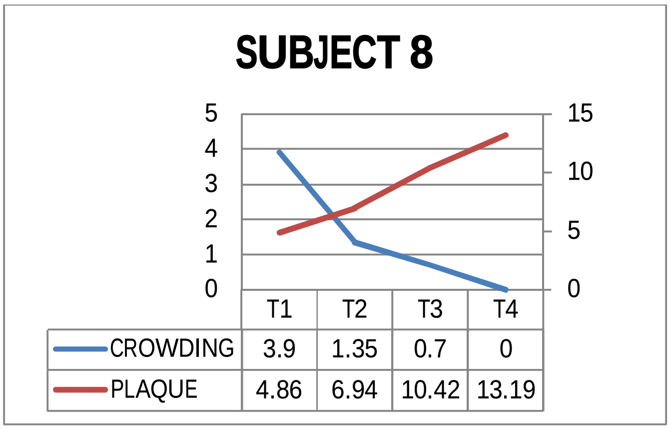


Figure 12.13: Patient 8 trends of plaque accumulation and anterior crowding over time

The trends for plaque and crowding are divergent suggesting that as the teeth became better aligned, the oral hygiene actually worsened.

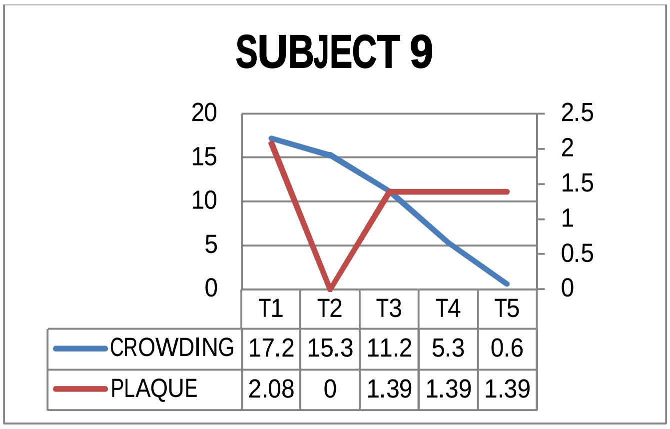


Figure 12.14: Patient 9 trends of plaque accumulation and anterior crowding over time

There was a consistent downward trend in irregularity for this patient whilst plaque scores were maintained at a fairly consistent, low level throughout.

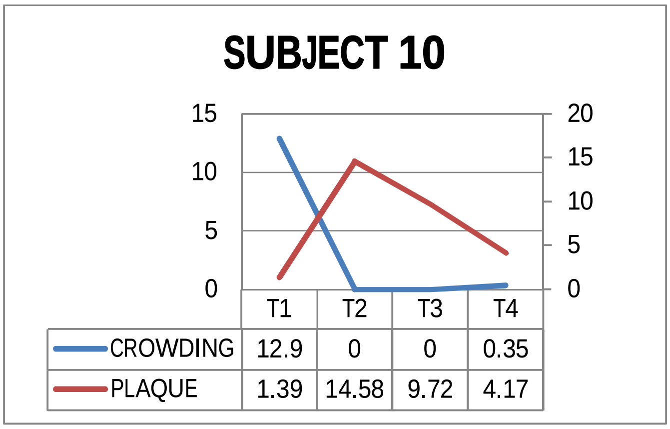


Figure 12.15: Patient 12 trends of plaque accumulation and anterior crowding over time

There was a marked improvement in the alignment of the teeth between the first and second appointments, however, the plaque accumulation increased at the second visit and had still not returned to the pre-treatment level by the fourth visit.

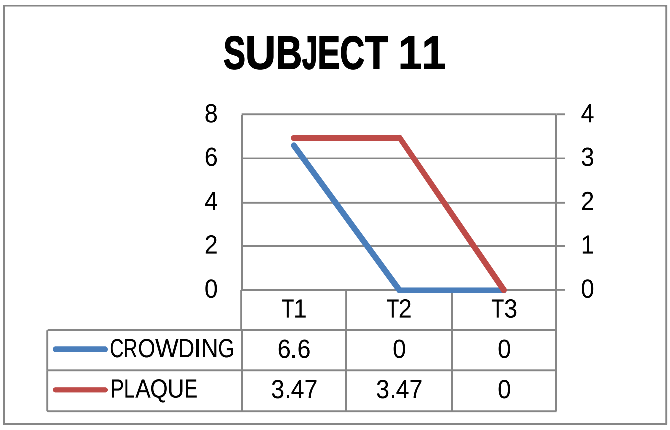


Figure 12.16: Patient 11 trends of plaque accumulation and anterior crowding over time.

Whilst both the plaque and crowding improved over the three visits, the changes were not in agreement as the oral hygiene improved only when the teeth had been aligned for a visit. The level of plaque accumulation, however, was very low at the start, therefore, the trend represents a very small change in amount of plaque present.

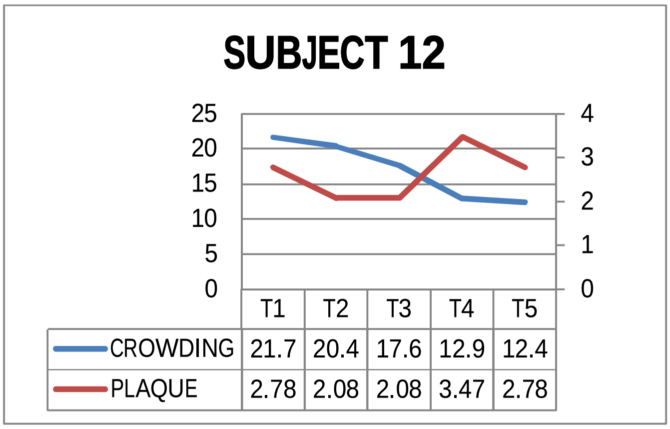


Figure 12.17: Patient 12 trends of plaque accumulation and anterior crowding over time.

The crowding improved by almost half over the five visits of observation, however, there was much less variability in the pattern of plaque deposits.

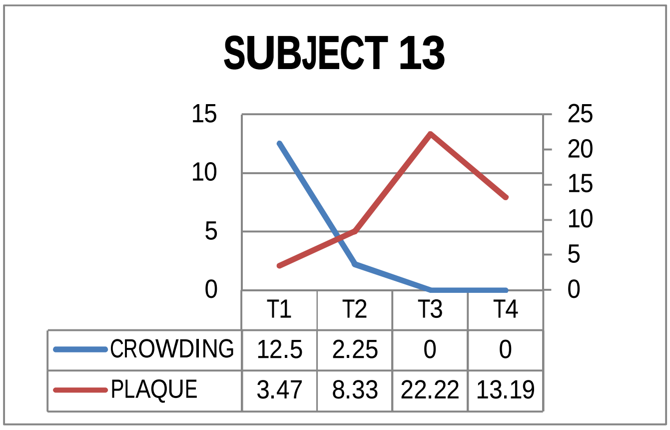


Figure 12.18: Patient 13 trends of plaque accumulation and anterior crowding over time.

Despite an improvement in irregularity there was a deterioration in oral hygiene over the first 3 visits. There was some improvement by the fourth visit but it did not improve to the level seen pre-treatment.